VOLUME 29 FEBRUARY 1951 NUMBER 2

# Canadian Journal of Technology

*Editor:* G. A. LEDINGHAM

NOTE:—This Journal prior to January, 1951, was Section F (Technology) of the Canadian Journal of Research.

*Published by* THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

## CANADIAN JOURNAL OF TECHNOLOGY

This was formerly *Section F, Canadian Journal of Research.* The change to the new name took place January 1, 1951. The CANADIAN JOURNAL OF TECHNOLOGY is published twelve times annually.

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Canadian Journal of Technology

*Issued by* THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 29 FEBRUARY, 1951 NUMBER 2

# A RAPID POLAROGRAPHIC DETERMINATION OF COPPER, CADMIUM, AND ZINC IN SILVER BASE ALLOYS[1]

BY E. G. FORD

## Abstract

Copper, cadmium, and zinc produce well defined polarographic waves in a supporting electrolyte of ammonia and ammonium chloride. A method has been developed for the simultaneous determination of these elements in silver base brazing solders with an accuracy of at least ± 1%, and, on a routine basis a sample may be analyzed for these components in less than a half-hour. The method is capable of extension to other similar materials containing these elements and nickel as well.

## Introduction

The polarographic behavior of copper, cadmium, and zinc in several supporting electrolytes has been thoroughly described (4). The ammonia – ammonium chloride system has been used successfully to determine these elements separately and together (5). Nickel also produces a good wave in that medium (3), and may be measured simultaneously with the others when present in similar concentrations. When copper, or another element whose discharge takes place before several others, is present in a relatively large amount, the "compensator technique" may be used unless succeeding waves are very small. In such a case, separations must be made prior to the polarographic procedure.

In silver-base alloys, especially those used for brazing purposes, copper, cadmium, and zinc may be present either separately or together as major components, their concentrations being generally of the same order of magnitude. Under these conditions, investigation has shown that the three elements can be readily determined on one polarogram.

## Apparatus and Reagents

This work was carried out with a Sargent Model XX visible recording polarograph, and water-jacketed cells that are described elsewhere (2). A quiet mercury pool, an external saturated calomel anode, and a lead wire anode wrapped about the capillary tube were all used. The latter was found most convenient for the purpose. Recalibrated Pyrex glassware and reagent grade

[1] *Manuscript received in original form June 1, 1950, and as revised, September 25, 1950. Contribution from Canadian Westinghouse Co. Ltd., Hamilton, Ontario, Research and Development Laboratories.*

chemicals were used throughout. A constant temperature of 25.00 ± 0.05°C. was provided by circulating water from a constant temperature bath. The capillary used had a rate of flow of 1.93 mgm. per sec. at 25°C.

## Procedure

To a 0.100 or 0.1000 gm. sample of silver-base alloy (depending on the accuracy required), add 10 ml. of 8 *M* nitric acid, and 5 ml. of 3 *M* sulphuric acid. Heat until vapors of sulphur trioxide begin to appear, then cool and dilute to 250 ml. in a volumetric flask, at 25°C. Mix and withdraw a 25.0 ml. aliquot sample by pipette into a 100 ml. volumetric flask, and add the following:

20 ml. of 5 *M* ammonium chloride,
20 ml. of 5 *M* aqueous ammonia,
5 ml. of 0.2% gelatin,
1 gm. of reagent grade sodium sulphite.

These reagents may be added by graduated cylinder, but all volumetric ware should be recalibrated.

Dilute to volume at 25°C., mix, and transfer a portion of the sample to a polarographic cell. Polarograph from −0.1 to −1.6 volts vs. S.C.E. at 25.0°C. at the maximum practical sensitivity, and measure the waves at −0.24 and −0.50 volts for the copper (II) and copper (I) complexes either separately or together. Measure the waves at −0.81 and −1.35 volts for cadmium and zinc respectively. When nickel is present, its wave may be measured at −1.10 volts. Convert the wave heights to current values and to grams or per cent either by direct calibration of the capillary with standard solutions or by reference to reliable published diffusion current constants. The pilot ion and standard addition methods of calibration may be used if necessary.

## Results and Discussion

Zinc and cadmium produce excellent waves in this medium. Copper produces a double wave which may be measured in total or on either half. In some cases the first wave of the copper complex tends to coalesce with the wave for mercury and may be difficult to measure. In this work the total wave as well as the second portion were separately measured, the double wave being used when possible. The residual current of the supporting electrolyte was accounted for in all calculations, but for practical routine work with brazing alloys, this was found to be so small that it could be disregarded, and measurements were taken simply through the principal slopes. A typical polarogram of a silver solder is shown in Fig. 1.

When the concentration of these elements is in the order of 20% or more, the number of figures obtainable by this polarographic technique is possibly less than might be given by gravimetric or titrimetric methods. Generally, for purposes of control or identification, an accuracy of from 0.5 to 1% of the component is all that is required, and this method is capable of that accuracy for rapid work.

Silver-base brazing alloys are generally soluble in nitric acid. Where tin is present it may be removed by filtration after dissolution of the other components. Nitric acid in excess is not desirable in the polarographic procedure,

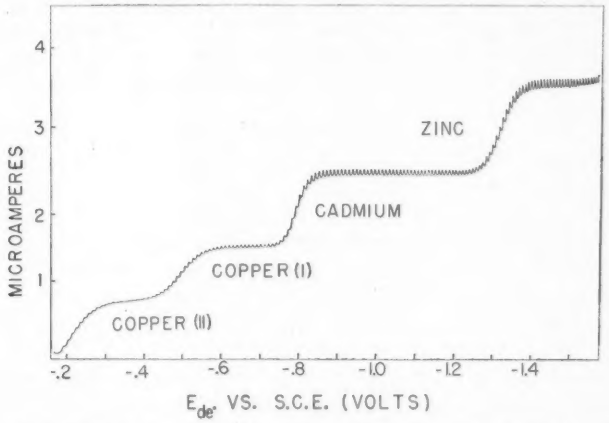


FIG. 1. *Polarogram of a silver base solder containing copper, cadmium, and zinc taken in 1 M ammonium chloride + 1 M aqueous ammonia.*

but may be removed by heating. In this work, a small amount of sulphuric acid was used to facilitate the removal of excess nitric and to control the final acidity of the solution to a relatively constant value. Gelatin was used as a maximum suppressor and sodium sulphite was used to remove oxygen. Oxygen may be removed by bubbling an inert gas such as nitrogen through the sample as is normally done in acidified solutions.

In this work, calibration was made from observed values found by use of standardized solutions of the three elements and pure silver (Table I). Lingane

TABLE I

CAPILLARY CALIBRATION FOR COPPER, CADMIUM, AND ZINC IN SILVER-BASE SOLDER USING 100 ML. AS THE BASIC SAMPLE VOLUME. DIFFUSION CURRENT CONSTANTS WERE CALCULATED

| Element | Concentration (mgm./l.) | Factor 1 (mgm./μa.) | Factor 2 (mgm./μa.) | Factor 3 (mgm./μa.) | Average (mgm./μa.) | Diffusion current constant |
|---|---|---|---|---|---|---|
| Zinc | 10.02 | $0.88_0$ | $0.87_8$ | $0.88_3$ | | |
| | 20.00 | $0.88_8$ | $0.87_9$ | $0.88_0$ | 0.880 | 3.98 |
| Cadmium | 28.40 | $1.56_9$ | $1.56_8$ | $1.57_7$ | | |
| | 56.70 | $1.57_2$ | $1.57_0$ | $1.57_2$ | 1.57 | 3.69 |
| Copper (1) | 31.90 | $1.72_5$ | $1.71_9$ | $1.71_8$ | | |
| | 16.01 | $1.72_1$ | $1.72_2$ | $1.71_6$ | 1.72 | 1.90 |
| Copper (total) | 31.90 | $0.86_2$ | $0.86_3$ | $0.85_9$ | | |
| | 16.01 | $0.85_9$ | $0.86_0$ | $0.85_6$ | 0.860 | 3.75 |

(4) reported diffusion current constants for these elements in the same supporting electrolyte (1 *M* ammonium chloride + 1 *M* aqueous ammonia). A comparison of calculated diffusion current constants with reported values was made. The values that were found agree quite closely with those reported by Lingane and would indicate that the presence of silver and other components has no effect on the diffusion currents. It would, therefore, be advantageous and advisable to use these constants rather than to make an empirical calibration.

Nickel is soluble in the supporting electrolyte and produces a wave just preceding that of zinc. It may be measured if present in the sample, but a better separation from zinc wave is obtained by reducing the ammonium chloride concentration to about 0.2 *M*. The sample size may be varied over a wide range by changing the dilution and size of the aliquot sample. It should be remembered, however, that excess acid will change the final ammonia concentration, which must be held within 10% of the recommended value.

An indication of the precision of measurement is given in Table II where samples of one solder were polarographed on three different days. A comparison of the average of these values with those taken by the methods of A.S.T.M. (1) is also made.

TABLE II

PRECISION OF MEASUREMENT ON ONE SOLDER SAMPLE ANALYZED ON THREE DIFFERENT DAYS, AND A COMPARISON WITH VALUES TAKEN BY THE METHODS OF A.S.T.M.

| Element | Day | Sample number | % found | Average, % | A.S.T.M. method, % |
|---|---|---|---|---|---|
| Copper | 1 | 1 | 18.6 | | |
| | | 2 | 18.7 | | |
| | 2 | 1 | 18.6 | | |
| | | 2 | 18.6 | | |
| | 3 | 1 | 18.7 | | |
| | | 2 | 18.6 | 18.6 | $18.7_2$ |
| Cadmium | 1 | 1 | 20.3 | | |
| | | 2 | 20.4 | | |
| | 2 | 1 | 20.3 | | |
| | | 2 | 20.4 | | |
| | 3 | 1 | 20.3 | | |
| | | 2 | 20.3 | 20.3 | $20.3_0$ |
| Zinc | 1 | 1 | 16.7 | | |
| | | 2 | 16.8 | | |
| | 2 | 1 | 16.7 | | |
| | | 2 | 16.7 | | |
| | 3 | 1 | 16.8 | | |
| | | 2 | 16.8 | 16.7 | $16.7_1$ |
| Silver | – | – | — | — | $44.1_6$ |

*Note: The deviation of the polarographic values from the average is somewhat less than may be expected for rapid control work. For most practical purposes an accuracy of 1% is sufficient. With reasonable care, measurement may be made to a precision of nearly ± 0.3%.*

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# THE DETERMINATION OF FAT AND SUGAR IN CHOCOLATE[1]

By E. E. Wood[2]


## Abstract

Methods for the estimation of the total fat and total sugar content of chocolate are briefly reviewed and compared. Detailed procedures for the rapid determination of both constituents from a single sample are presented. In principle, these are based upon rapid extraction of the fat with solvent and separation of the extract by centrifuging. The defatted residue is combined with a definite proportion of water and the sugar content of the liquid measured by the refractometer.


## Introduction

In establishing the composition of chocolate products, determinations of the important constituents fat and sugar are most frequently required. It is therefore highly advantageous to have available convenient, direct, and rapid procedures for the quantitative determination of these constituents. The present methods have been developed as a contribution to the rapid estimation of total fat and total sugar in cacao products, such as,—cocoa, coating chocolate, milk chocolate, and a variety of chocolate confections.

## Determination of Fat

*Established Methods*

The familiar Soxhlet Extraction or the Knorr Extraction procedures are official methods (1, p. 229) for the determination of fat in cacao products. However, there are some practical difficulties encountered in carrying out the tests. Owing to the exceedingly fine grinding during manufacture of these products, much of the fibrous cacao material is very finely divided, and some may pass through even the best of extraction thimbles in the Soxhlet extractor. Furthermore, as the extraction proceeds, the powdered sample packs together, slowing down the process to such an extent that many hours of continuous extraction are required to make certain that the fat is completely extracted.

In the Knorr method the sample is extracted repeatedly with portions of solvent in a special extraction tube fitted with a filter mat of packed asbestos, filtration being promoted with the aid of vacuum. Again the procedure is attended by some difficulties arising from much the same causes as those encountered in the Soxhlet extraction—passage of fine particles, slow filtration, and eventual packing of the sample.

Another type of procedure which has been utilized in the chocolate industry in recent years is the use of methods based upon measurement of the refractive

[1] *Manuscript received August 29, 1950.*
*Presented at the Annual Conference of the Chemical Institute of Canada, Analytical Chemistry subject division, Toronto, June 22, 1950.*
[2] *Research Chemist, Consumers Research Laboratories Ltd., Toronto.*

index of a solution of the fat in specially selected solvents. Representative of this class of methods is the procedure described by Stanley (6), in which the fat is extracted by tricresyl phosphate under specified conditions, the solution filtered, its refractive index measured, and the fat content derived from calibration curves. Such a method based upon the indirect and arbitrary relation between composition and physical property is of greatest value applied to the rapid routine control of familiar products but has considerably less utility for products of unknown composition and manufacture.

The measurement of the density of a solvent extract of the fat by means of a special hydrometer forms the basis for another indirect determination and the method of Harris (3) is representative.

The principle of simple extraction by mixing the product with solvent followed by separation of the mixture through filters or by centrifuging has been used in the industry for a long time as the basis for methods of fat analysis under a variety of differing details. These procedures have remained largely unpublished but the method of Hughes (4) may be cited as typical. In this connection, while the present manuscript was in the course of preparation, details of the centrifuge method for fat extraction were published by Kobe (5).

In the present method about to be described, advantage is taken of the fact that owing to the processes to which the product is subjected during manufacturing the sample material is in a state of very fine division and readily undergoes thorough dissolution in solvent. In principle the method depends upon dissolution of the sample and extraction of the fat by petroleum ether, followed by separation of the solid residue from the fat-ether solution by means of centrifuging. The solvent extract is decanted to a tared flask and the fat recovered for weighing after removal of the solvent by evaporation.

## Procedure for Determination of Fat

*Reagents and Apparatus*

(1). Petroleum ether—boiling range, 40° to 60°C. Reagent grade, redistilled so that it leaves no residue on evaporation.
(2). Centrifuge tubes—50 or 100 ml. capacity, round bottom, with pouring lip.
(3). Flasks–Soxhlet extraction flasks, 250 ml. with 24/40 standard taper joint.

*Sample Preparation*

All types of chocolate should be reduced to small particles by chopping or preferably by grating. Samples of cocoa powder are used directly. Owing to the methods of manufacture, chocolate products are extremely uniform and homogeneity of sampling is assured.

*Procedure*

Weigh 5 gm. of the sample into a centrifuge tube, add about 35 ml. of petroleum ether, and stir the mixture thoroughly with a glass rod to extract the fat. A slight amount of solid residue will remain coating the stirring rod; retain the rod for further use.

Place a rubber cap over the tube to prevent evaporation and spin the tube in a centrifuge at about 2500 r.p.m., for 10 min.; there should be produced a lower firmly packed layer of solid residue and a clear upper layer of solvent solution of fat. Decant the solvent directly into a flask which has been previously dried in the oven and weighed.

Add a second portion of about 30 ml. of petroleum ether to the tube and mix the solvent and residue thoroughly with the same stirring rod used before. Centrifuge for about five minutes at about 2000 r.p.m. Again decant the clear layer to the previously collected extract in the flask. Repeat the extraction with a third portion of solvent, centrifuge, and combine the extracts in the flask. (Save the cacao residue in the centrifuge tube and the stirring rod for the subsequent determination of sugar.)

Evaporate the solvent from the fat extract. It is preferable to remove the last traces of solvent from the fat by attaching the flask to a simple distilling unit with vacuum, the flask being surrounded by a beaker of boiling water. Dry the flask thoroughly and weigh to obtain the fat content of the sample.

*Notes on the Procedure*

(1). The first extraction may be a little difficult to clarify completely owing to the viscosity of the concentrated fat solution. Add a little more of the ether, gently stir the supernatant liquid, centrifuge again, and then decant.

(2). The variation among replicate determinations has been found to be $\pm$ 1% fat and the accuracy of the method is equal to that obtained with the Soxhlet extraction method.

(3). Three extractions have been found to give essentially complete extraction, sufficient for all practical purposes.

## Determination of Sugar

*Established Methods*

The accepted method for the determination of sucrose in plain chocolate is that of the Association of Official Agricultural Chemists (1, p. 234). The sucrose is determined through polarization of the defatted residue after extraction with water and suitable clarification. The "direct" and "invert" polarizations are measured in a manner analogous to the principles widely employed in the analysis of various sugars by polarimetric methods. By means of a set of specific equations the sucrose content of the chocolate may be calculated from the polarization data. The accuracy of the method is good but it is complicated and slow, thus being quite deficient as a means of rapid analysis of composition.

In the case of milk chocolate products there is also an official method applicable to lactose (1, p. 233) specifically. The clarified filtrates prepared for the sucrose determination are used and reducing sugars are determined by copper reduction according to the method of Munson and Walker. Owing to the fact that the presence of sucrose influences the reducing value, the amount of

reduced copper must be corrected for sucrose by means of an empirical equation involving the sucrose polarization data to yield a preliminary approximate lactose value. Then, from this estimated polarimetric sucrose/lactose ratio and the total copper, the sucrose correction is computed and the net amount of reduced copper is used with the regular Munson and Walker table for lactose. Although these methods are reasonably specific for the various sugars involved, it is evident from the preceding review that the procedures are much too complicated and time consuming for rapid and convenient control of the analytical composition of chocolate products. On the other hand, the method about to be described is a very simple procedure for the determination of total sugars. It makes use of the fact that all the common sugars in aqueous solution have almost identical indexes of refraction at the same concentration. The utilization of this principle confers unique simplification considering the kinds and combinations of sugars which may be encountered in different chocolate products. For example, milk chocolate would contain lactose from the milk as well as sucrose, while plain chocolate would ordinarily contain sucrose alone and moreover either type might contain added dextrose (corn sugar). In any case the amount of total sugars is valuable information and usually sufficient.

## Procedure for Determination of Sugar

*Reagents and Apparatus*

(1). Basic lead acetate--Horne's sugar reagent as specified for sugar analysis by polarimetry.

(2). Refractometer—Abbe type, scaled in per cent sugar by weight at 20°C. (degrees Brix).

*Sample Preparation*

The material must be initially defatted and it is particularly convenient to use the residue remaining in the centrifuge tube from the previous fat analysis. Drive out the remaining ether by warming the tube in hot water and stirring up the residue with the glass rod.

*Procedure*

Add a definite weight of water, 15 gm., to the dry residue in the tube. This is most readily accomplished volumetrically by means of burette or pipette. Mix the residue and water thoroughly with the rod, taking care to get an even suspension and complete solution of the sugar. Add just sufficient dry basic lead acetate to clarify the suspension. The amount is not highly critical but more than a slight excess should be avoided, owing to the possibility of raising the refraction. Different types of chocolate may require different amounts of the lead to coagulate the nonsugar, refractive, soluble extractives; about 0.12 gm. is average for plain chocolate and 0.25 gm. for milk chocolate.

The mixture is settled for a few minutes or a small portion filtered through a coarse paper or preferably given a slight centrifuging. The separation need only be partially complete, adequate for the liquid to give a sharp image in the

refractometer. Using a dry tube or pipette, transfer a few drops of the liquid taken from well below the surface to the prism of the refractometer and measure the refraction (sugar scale) and the temperature of the prism.

*Calculations*

$$S = R + C$$

$S$ — is the weight per cent sugar in the liquid.

$R$ — is the refractometer reading at the temperature of measurement.

$C$ — is the temperature correction to be applied algebraically to the reading $R$ for compensation to the instrument calibration at 20°.

The value of $C$ also varies with concentration and suitable correction tables are found in various handbooks (1, p. 827; 2) supplying data for sugar analysis.

The sugar content of the chocolate is computed as follows,—

$$\% \text{ Sugar} = \frac{S}{(100 - S)} \times \frac{\text{weight of water}}{\text{sample weight}} \times 100.$$

Since the sample weight is ordinarily 5 gm., and the weight of added water is 15 gm., this reduces to,—

$$\% \text{ Sugar} = \frac{S \times 300}{(100 - S)}.$$

*Notes on the Procedure*

The method is believed to be accurate to about ± 0.6% total sugar in plain chocolate. In the case of milk chocolate the error is likely somewhat greater but not definitely determinable owing to uncertainties as to the accuracy of other methods which can be used for comparison. These limits of analytical precision are adequate and acceptable for production control and purchase specifications in the chocolate industry.

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# CANADIAN ERUCIC ACID OILS

## VI. BLOWING OF RAPESEED, MUSTARD SEED, AND WEED SEED OILS[1]

By N. H. Grace[2] and A. Zuckerman[2]


### Abstract

Crude, solvent extracted weed seed oil could be air blown at 266°F. without preliminary treatment, whereas crude, hot pressed mustard or rapeseed oils frothed excessively unless pretreated by one of the following: bleaching, alkali refining, water washing to remove lecithin, or flash heating at 383°F. Increases in refractive index, density, F. F. A., and viscosity after blowing for 6, 12, 18, 24, and 36 hr. were generally similar for all oils, but alkali refined oils showed a slower increase in refractive index and F. F. A., and a slower decline in iodine value than did the corresponding crude or bleached oils. Blowing for six hours increased light transmission at 440 and 660 mμ; further heating decreased transmission at the lower wave length and the value attained after 24 or 36 hr. heating approximated that of the untreated oil. Lecithin free and flash heated rapeseed oils showed the least change in transmission over the range of heating periods. Flash heated rapeseed oil increased greatly in F. F. A. and attained the lowest iodine value and highest viscosity (1931 S. U. sec. in 36 hr.).

Miscibility in red paraffin oil decreased with blowing; weed seed oil was inferior to mustard seed oil, with rapeseed oil giving the best miscibility. Alkali refined and lecithin free rapeseed oils showed better miscibility than the corresponding bleached or flash heated oils.

Blown weed or mustard seed oils dissolved in ethyl acetate tolerated addition of more absolute ethanol without separation than did blown rapeseed oils; alcohol toleration tended to decrease with extent of blowing. Toleration of denatured ethanol was less than half that for absolute ethanol. Esterification of part of the F. F. A. content with ethanol or glycerol improved alcohol toleration.


### Introduction

In Europe and Asia erucic acid oils have been extensively used for edible purposes, and rather wide variations in certain properties, such as unsaturation, could be expected to have relatively little significance. In North America, erucic acid oils are used primarily for industrial purposes such as the formulation of lubricants and plasticizers, and the more exacting requirements of these specialized uses cannot tolerate wide variations in properties of the oil.

Canadian production of rapeseed oil (Argentine rape, *Brassica campestris* L. var. *Napus*) has recently been expanded, and expressed mustard seed oil and solvent extracted weed seed oil (largely from mustard seed screenings) have also become available. While there is a general similarity in properties, the iodine values of these oils range from about 103 to 124. Native rapeseed oil has required alkali refining and bleaching to avoid violent frothing on blowing. Simpler preblowing treatment could lead to greater use of native erucic acid oils for the preparation of blown oils.

[1] *Manuscript received June 26, 1950.*
*Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 100 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2306.*

[2] *Chemist.*

Air blown or oxidized erucic acid oils find wide industrial application (2, 7, 9). They are prepared by passing air through agitated oil at 248°–392°F. (2, 3, 4, 7). On blowing, oils increase in both density and viscosity; the common vegetable oils approach castor oil in these respects, but are more miscible with mineral oil, and less soluble in ethyl alcohol.

The variation in unsaturation of native erucic acid oils suggests that the blown oils might differ in applicability to particular industrial uses. The purposes of this investigation were: (1) to develop simplified pretreatments which would permit blowing without excessive refining loss, (2) to modify the properties of blown rapeseed oil, thereby permitting a wider use in formulations requiring a greater alcohol solubility, and (3) to show gross differences between types of erucic acid oils. Direct comparisons are made between alkali refined rapeseed and weed seed oils, and similarly bleached rapeseed and mustard seed oils.

## Materials and Methods

Rapeseed and mustard seed oils (5), hot pressed by Prairie Vegetable Oils Ltd., Moose Jaw, Sask., and weed seed oil (6), solvent extracted by Edible Oils Ltd., Fort William, Ont., from weed seed screenings comprised largely of wild mustard seed, were used because they represent commercially available erucic acid oils. The effects of various refining and preblowing treatments were studied with a series of nine oils. The pretreatments were selected for study as representing minimum processing effort and material loss with freedom from frothing on blowing. The various treatments given these oils are set forth in Table I, with some data descriptive of the physical and chemical pro-

TABLE I

CHARACTERISTICS OF OILS BEFORE BLOWING

| Number | Description | Refractive index at 77°F. | Relative trans., % at | | Iodine value, cgm./gm. | F.F.A., % as oleic |
|---|---|---|---|---|---|---|
| | | | 440 mμ | 660 mμ | | |
| | Weed seed oil | | | | | |
| 1 | Crude | 1.4728 | 5.0 | 31.8 | 123.8 | 1.51 |
| 2 | Bleached with 4% superfiltrol at 212°F. | 1.4729 | 25.0 | 88.9 | 125.0 | 1.20 |
| 3 | Alkali refined | 1.4728 | 5.0 | 51.0 | 124.9 | 0.06 |
| | Rapeseed oil | | | | | |
| 4 | Bleached with 4% superfiltrol at 392°F. | 1.4713 | 12.0 | 90.4 | 101.8 | 1.40 |
| 5 | Alkali refined | 1.4702 | 5.0 | 48.1 | 103.9 | 0.05 |
| 6 | Alkali refined, bleached, slightly hydrogenated | 1.4700 | 40.0 | 89.5 | 99.1 | 0.18 |
| 7 | Crude, water-washed to remove lecithin | 1.4704 | 4.9 | 19.9 | 104.8 | 0.60 |
| 8 | Crude, heated to 383°F. for 5 min., cooled to 266°F., then blown | 1.4702 | 4.2 | 6.2 | 101.5 | 0.76 |
| | Mustard seed oil | | | | | |
| 9 | Bleached with 4% superfiltrol at 392°F. | 1.4720 | 16.2 | 94.5 | 109.5 | 2.65 |

perties prior to air blowing at 266°F. The oils were refined and bleached by standard methods as previously described (5). Rapeseed and mustard seed oils were bleached at 392°F. because lower temperatures yielded oils which frothed violently.

The temperature of 266°F. was selected for blowing since it effected appreciable thickening in a reasonable period of time. Samples of 200 gm. each were blown in a 500 ml. three-neck flask in a heating mantle controlled by a variable transformer. A fritted glass disk dispersed the entering air stream. The air stream was regulated by a flow meter at two liters per minute and passed through water in a gas washing bottle maintained at a temperature of about 68°F. to provide a partial pressure of 17 mm. water vapor.

All oils were blown for 6, 12, 18, and 24 hr.; in addition lecithin free and flash heated rapeseed oils (oils 7 and 8 in Table I) were also given a 36 hr. treatment. The temperature was maintained at 266°F.± 9°F.

The refractive indices, specific gravities, free fatty acid contents, and Wijs iodine values were determined by A.O.C.S. methods; the viscosity was measured in the Saybolt Viscosimeter and is recorded in S. U. seconds at 210°F. The relative transmissions at 440 and 660 mμ were measured on an Evelyn photoelectric colorimeter using Stanolax as a standard.

Miscibility of blown oils with No. 5 red paraffin oil* (Canadian Oil Companies Ltd.) was determined using four blends of mineral and blown oil in the following proportions by weight: 95:5; 90:10; 85:15; and 80:20. The mixtures were agitated and warmed to 120°F. to ensure solution, transferred to 4-oz., clear glass bottles, and allowed to stand at laboratory temperature (75°F.) for 24 hr. before they were examined. Blends were then graded as clear, hazy, or showing definite separation. They were then cooled in an ice water bath to 35°F. for a period of about one and one-half hours and the condition recorded again.

The use of blown oils as plasticizers, such as with nitrocellulose coatings in the preparation of artificial leathers, is limited by solubility in common solvent mixtures. Consequently alcohol solubility for both absolute ethanol and denatured alcohol D. A. G. No 2–D. (ethanol 65 O. P., 90 gal.; wood alcohol, 9

* *CHARACTERISTICS OF NO. 5 RED PARAFFIN OIL*

| | |
|---|---|
| *Gravity, ° A. P. I.* | *23.5–24.5* |
| *Specific gravity at 60° F.* | *.9129–.9071* |
| *Flash point, °F. (Cleveland Open Cup)* | *415–420* |
| *Fire point, °F. (Cleveland Open Cup)* | *475–490* |
| *Viscosity, Sayholt Universal seconds at 100° F.* | *300–310* |
| *Viscosity, kinematic centistokes at 100° F.* | *65.0* |
| *Viscosity, Sayholt Universal seconds at 210° F.* | *50–52* |
| *Viscosity, kinematic centistokes at 210° F.* | *7.29–7.9* |
| *Pour point, °F.* | *30–35* |
| *Color, A.S.T.M. arbitrary units* | *4½–6½* |
| *Conradson carbon, %* | *0.37–0.3* |
| *Neutralization No., mgm. KOH per 1 gm. sample* | *0.04* |

TABLE II

EFFECTS OF REACTION WITH GLYCERINE AND ALCOHOL ON THE PROPERTIES OF BLOWN OILS

| No. | Treatment | Universal Saybolt viscosity, sec. (210°F.) | Iodine value, cgm./gm. | Free fatty acid as oleic, % | Relative transmission, % at | | Ethanol, gm. to precipitate 3 gm. blown oil dissolved in 15 gm. ethyl acetate | | Refractive index at 77°F. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 440 mμ | 660 mμ | Absolute | Denatured | |
| I | Alkali refined bleached 2% superfiltrol rapeseed oil (500 gm.) blown 26 hr. at 5 l./min. air | 632 | 41.8 | 3.46 | 6.0 | 64.0 | 26.8 | 16.1 | 1.4744 |
| *a* | No. I reacted with twice theoretical amount glycerine | – | 45.6 | 6.68 | 3.5 | 1.1 | 25.4 | 11.7 | 1.4779 |
| *b* | No. I reacted with four times theoretical amount glycerine | 626 | 50.7 | 6.94 | 3.6 | 1.8 | 24.7 | 13.8 | 1.4780 |
| *c* | No. I refluxed with 10 times theoretical amount absolute ethanol | 173 | 51.1 | 7.36 | 0.9 | 0.0 | * | 34.8 | 1.4752 |
| *d* | No. I reacted with four times theoretical amount of glycerine and 0.3% stannous chloride | 545 | 47.4 | 4.78 | 2.1 | 0.0 | * | 21.1 | 1.4808 |
| *e* | No. I in xylene solution reacted with four times theoretical amount glycerine and 0.3% stannous chloride, 2.1 cc. water collected in attached water trap from 70 gm. blown oil in 24 hr. | 703 | 42.0 | 2.72 | 3.0 | 1.1 | 29.2 | 15.1 | 1.4797 |
| II | Bleached crude mustard seed oil blown 24 hr. at 266°F. 6 l./min. air | 1065 | 49.1 | 6.59 | 5.8 | 55.0 | 24.7 | 11.6 | 1.4780 |
| *a* | Acids prepared from II (acid equiv. wt. 3141) reacted with equiv. wt. glycerine | 42 | 54.1 | 7.98 | 0.1 | 0.0 | * | * | 1.4665 |
| *b* | Oil II*a* reacted with four times theoretical amount of glycerine and 0.3% stannous chloride | 64 | 49.2 | 3.60 | 0.7 | 1.0 | * | * | 1.4722 |
| III | Commercially blown rapeseed oil | 850 | 55.3 | 4.31 | 0 | 4.3 | 11.3 | 6.4 | 1.4799 |

* *No clouding on addition of 120 gm. alcohol.*

gal; and benzine, 1 gal.) was determined by dissolving 3 gm. blown oil in 15 gm. of ethyl acetate and titrating with the alcohol until a permanent cloud was formed, the titration being made at room temperature of about 75°F.

Blown oils were subjected to various chemical treatments in an effort to increase the alcohol toleration. These experiments included esterification with glycerol and ethyl alcohol and the action of hydrogen peroxide prior to blowing. Details of some of these experiments are set forth in Tables II and III. Re-

TABLE III

MODIFICATION OF THE PROPERTIES OF BLOWN RAPESEED OILS BY REACTION WITH ETHANOL

| Blown oil | Treatment | Viscosity, S.U. sec. 210°F. | F.F.A., % as oleic | Weight, gm. ethanol to precipitate 3 gm. oil dissolved in 15 gm. ethyl acetate | |
|---|---|---|---|---|---|
| | | | | Absolute | Denatured |
| Rapeseed No. 1 | No further treatment | 908 | 6.4 | 18.0 | 10.3 |
| | Refluxed 12 hr. with 5 times amount of absolute ethanol equivalent to F.F.A. | 590 | 5.5 | 31.4 | 14.9 |
| | Refluxed 12 hr. with 10 times amount of absolute ethanol equivalent to F.F.A. | 588 | 5.5 | 23.5 | 11.6 |
| Rapeseed No. 2 | No further treatment | 1931 | 11.2 | 23.2 | 12.5 |
| | Refluxed 12 hr. with 10 times amount of absolute ethanol equivalent to F.F.A. | 788 | 8.9 | 39.6 | 18.5 |
| Commercial blown cottonseed oil | No further treatment | 311 | 5.1 | 46.3 | 18.1 |

actions with glycerol were attempted at a pressure of about 10 mm. and a temperature of about 284°F. for three hours in a vessel with a 12-in. Vigreux column attached. On a few occasions, when stannous chloride had been used as catalyst, the reaction mixture was taken up in diethyl ether, washed with dilute sodium bicarbonate solution, dried over sodium sulphate, and the solvent removed.

In one experiment (II*a*, Table II), acids were prepared from blown mustard seed oil by addition of hydrochloric acid to soaps prepared by sodium methylate solution. These acids were subsequently reacted with glycerol. The amount of glycerol used in this and other reactions was based on the free fatty acid content of the blown oil determined by analysis.

Lecithin free rapeseed oil was treated with 30% hydrogen peroxide at rates of 2% and 4% peroxide per 100 gm. oil, addition being made in the presence

of about 1% glacial acetic acid, and the mixture was stirred at 122°F. for three hours. The temperature was then raised gradually to 266°F. when blowing commenced. Further experiments used 90% hydrogen peroxide at the rate of seven parts per 100 parts of oil, and the mixture was stirred for one hour and then the temperature was gradually raised during the second hour to the blowing temperature of 266°F.

## Results

Crude solvent extracted weed seed oil could be blown without any refining treatment whereas crude expressed rapeseed and mustard seed oils frothed excessively. These expressed oils could be blown after bleaching at 392°F. or after refining or other preblowing treatment such as removal of lecithin or flash high temperature treatment.

*Refraction, Density, and Viscosity*

Fig. 1 describes the changes in refraction, density, and viscosity of the oils on treatment. The responses of weed seed oil were more marked than those of mustard seed or rapeseed oils. Alkali refined oils tended to respond somewhat

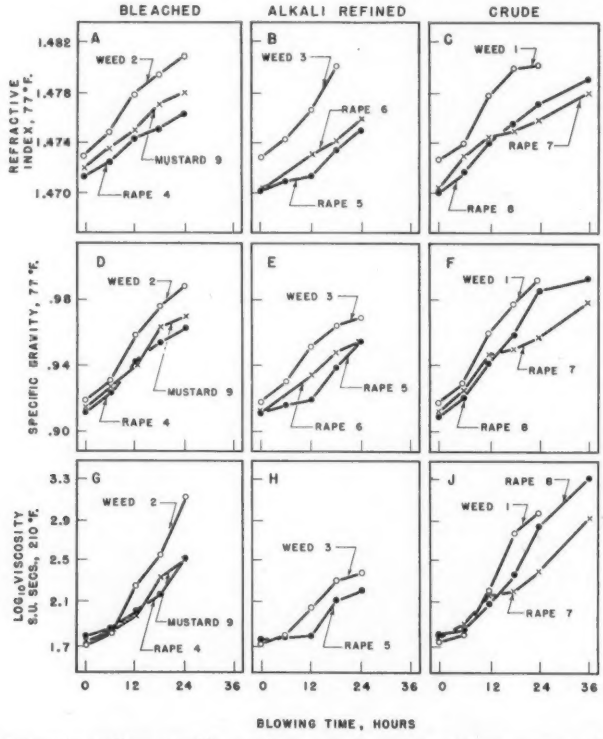


FIG. 1. *Refractive indices, specific gravities, and viscosities of blown erucic acid oils (see key in Table I).*

more slowly. Bleaching crude rapeseed oil for 20 min. with 4% Superfiltrol raised the initial refractive index when compared with alkali refined, lecithin free, or flash heated rapeseed oils. Mustard seed oil was more nearly like rapeseed oil than like weed seed oil. Values for the viscosity of oil 6 were between the values for oils 3 and 5 (Fig. 1, H).

*Color Change*

Changes in relative transmission at 440 and 660 mμ of oils on blowing are described in Fig. 2, A–E. Initial values were in general agreement with earlier

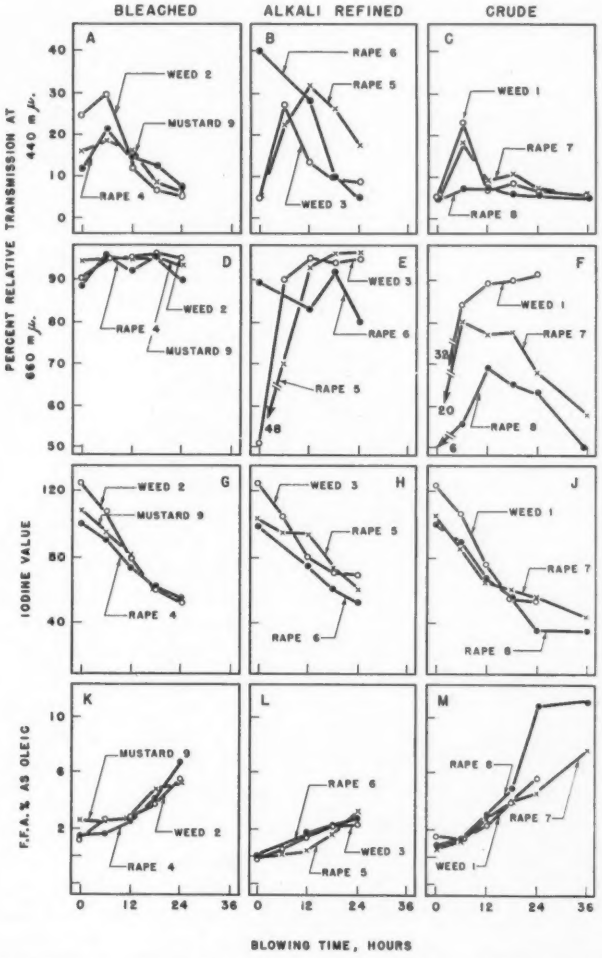


FIG. 2. *Transmission, relative to Stanolax, at 440 and 660 mμ; iodine values; and free fatty acid contents of blown erucic acid oils (see key in Table I).*

findings (5). A six hour period of blowing generally effected increase in transmission (decrease in color) at 440 mμ; on further blowing, transmission declined. Bleached oils (D) at 660 mμ showed high initial transmission which varied only slightly over the range of blowing periods. Relative transmission after 24 hr. heating was about the same for bleached and crude oils (A, C) at 440 mμ, with marked differences shown at 660 mμ.

*Iodine Values and Free Fatty Acid Contents*

Fig. 2, G–M, describes changes in iodine values and free fatty acid contents. These data demonstrate the similarity of the oils. Iodine values for bleached oils (G) fell to about the same value after 18 and 24 hr. blowing, even though the initial values differed appreciably. Blowing effected a progressive increase in free fatty acid content which depended somewhat on the initial acid content. Flash heating of crude rapeseed oil (M, oil 8) had little effect on the initial acid content (0.74%) but subsequent increase was marked, suggesting that thermal degradation of froth-forming material is associated with some fatty-acid-forming promoter.

*Precipitation of Blown Oils from Ethyl Acetate Solutions by Absolute and Denatured Ethyl Alcohols*

Fig. 3, A–F, shows solubility relationships for blown oils and ethyl alcohols. The weight of alcohol required to cloud a solution of 3 gm. blown oil dissolved in 15 gm. ethyl acetate is plotted against blowing time. Initially, all the oils, excepting flash heated rapeseed oil (C, oil 8) failed to cloud when 120 gm. of absolute alcohol was added; this condition held for alkali refined oils blown for six hours (B). It is apparent that the tolerance is much lower with denatured ethanol, D. A. G. No. 2–D (Fig. 3, D–F).

Generally, the amount of alcohol required to effect clouding decreased with the extent of blowing. Alkali refined oils (B, oils 3 and 5) tolerated substantially more absolute ethanol than comparable bleached or crude oils. However, reference to Fig. 1, H, suggests that this condition may relate to the somewhat lower viscosities attained. Nevertheless, the relation between alcohol miscibility and viscosity is hardly direct, since 24-hr. heated oils 1 and 2 gave relatively high alcohol values (Fig. 3, A, C) in conjunction with high viscosities (Fig. 1, G, J). Rapeseed oil, whether bleached, alkali refined or crude, showed consistently poorer alcohol toleration than weed seed oil. Lecithin free and flash heated crude rapeseed oils (Fig. 3, C, F) showed marked overall improvement in denatured alcohol toleration on heating, while the absolute ethanol toleration varied little over the range of heating treatments.

Flash heated crude rapeseed oil (Fig. 3, F, oil 8) tolerated 14 and 12.5 gm. denatured ethanol when blown 24 and 36 hr. respectively. Commercially blown rapeseed oil (Table II) with a viscosity of about 850 S. U. sec., gave alcohol values of 11.3 gm. absolute ethanol, and 6.4 gm. for denatured ethanol.

*Paraffin Miscibility*

Observations on the miscibility of blown oils and No. 5 red paraffin oil after standing 24 hr. at 75°F., and then held for about 90 min. at 35°F., are given in

Appendix Table I. Variations existed in miscibility attributable to the kind of oil, preblowing treatment, and extent of blowing. An arbitrary scoring system (clear blends scored 0, hazy blends 1, and separated blends 3) was applied to the observations of Appendix Table I, to obtain average scores. Increasing departure of score from zero, the ideal condition, indicates decreasing miscibility of blown oil in mineral oil.

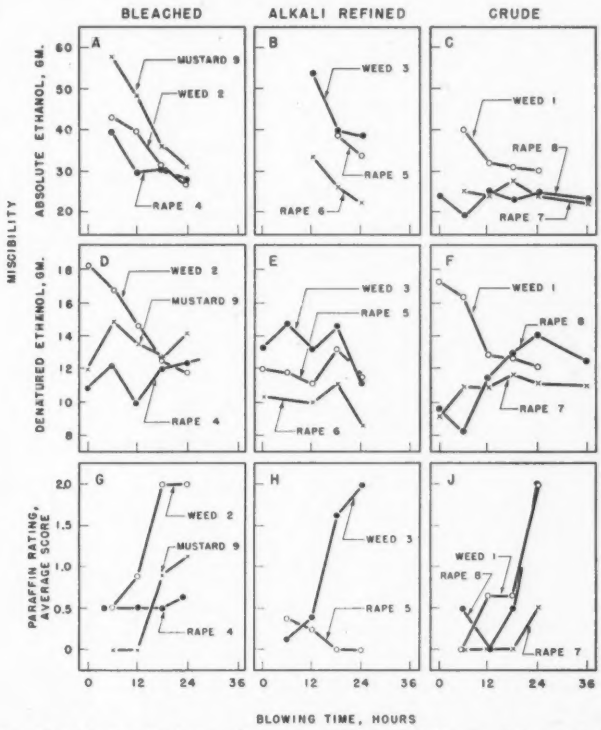


FIG. 3. *Precipitation by absolute and denatured ethanol of blown oil from solution in ethyl acetate; average arbitrary scores for miscibility of blown oils with mineral oil (see key in Table I).*

In Fig. 3 (F, H, J), scores for miscibility averaged over the four blends and two temperatures (75°F. and 35°F.) of observation are plotted against blowing time. Weed seed oil shows consistently high average scores indicating relatively poor miscibility with paraffin oil. Rape oils, bleached, alkali refined, or lecithin free were consistently low in score, indicating good blending property.

Blends with 10%, 15%, or 20% blown oil were closely similar in score; the blends with 5% blown oil and 95% mineral oil had the highest scores.

*Chemical Treatment of Blown Oils*

The effects of glycerol and ethanol treatment of blown oils on their alcohol toleration and other properties are indicated in Table II. Rapeseed oil I (Table

II) was blown to a S. U. viscosity of 632 sec. and portions of this oil were subjected to various (I*a*-I*e*) treatments. A feature of the results was the low relative transmissions of all the products (dark color). However, they did not differ widely from a commercially blown oil, with a S. U. viscosity of 850 sec. Alcohol toleration was increased by reacting blown oil with glycerol in the presence of stannous chloride as catalyst. A striking increase in alcohol toleration followed reaction of blown oil with ethanol.

Further effects of alcohol reaction with two blown rapeseed oils (S. U. 908 and 1931 sec. respectively) are described by the data of Table III. When oils were reacted for 12 hr. with five to 10 times the amount of absolute ethanol equivalent to the free fatty acid content, and unreacted alcohol removed, the resulting oils had reduced viscosity but improved alcohol toleration. These results, and also those with oil I*c* of Table II, show that this mode of treatment yields blown rapeseed oils with alcohol toleration characteristics more nearly like those of commercial blown cottonseed oil (Table III). Only a partial reaction of free fatty acid groups with ethanol or glycerol is effected, suggesting that the acid groups involved are constituents of the complex oxidized molecular structures. Indeed, in some instances the acid content increases on attempted esterification, indicating possible change in molecular structure. The latter effect may involve some scission at oxidized double bonds under esterification conditions.

Table II also shows that fatty acids prepared from a blown mustard seed oil (II) S. U. viscosity 1065 sec. after heating with glycerol yields a mobile oil (II*a*) viscosity 42 S. U. sec., with high toleration for alcohol. Further esterification with glycerol in presence of stannous chloride (II*b*), reduced the free fatty acid content to 3.6%, increased the viscosity to 64 S. U. sec., about that of unblown oil, but maintained extremely high alcohol miscibility. Apparently, saponification of the viscous blown oil breaks down the polymeric structures which are not reformed on re-esterification with glycerol.

Sodium bicarbonate washing of an ethereal solution of blown oil reduced the F. F. A. from 7.74% to 5.76%, and increased toleration by 7% for denatured alcohol and 22% for absolute ethanol.

*Effects of Hydrogen Peroxide Treatment*

Treatments of lecithin free rapeseed oil with 10% or 30% hydrogen peroxide prior to blowing had little effect on the alcohol toleration of the resulting oil over the viscosity range from 105 to 1214 S. U. sec. though there was slight improvement in toleration with oils in the 100 sec. S. U. viscosity range. However, a marked increase in rate of thickening was observed. Oil treated with 7% of 90% peroxide attained in 6 and 18 hr. blowing viscosities of 105 and 514 S. U. sec., control oils had viscosities of 77 and 158 sec. Peroxide treatment did not improve color of the blown oil as judged by relative transmission measurements, but markedly increased the free fatty acid content. This increase could

be due to either hydrolysis of glycerides or scission at double bonds. Peroxide treated oils contained a small amount of suspended solid matter that was insoluble in petroleum ether, indicating the formation of some heavily oxidized or hydroxylated material on blowing.

## Discussion

The extent of refining affected the behavior on blowing and the resulting properties of the blown oil. Color of dark oils was reduced during the early stages of blowing, though longer treatments tended to yield products similar in color to the oil before blowing; but alkali refined oils remained substantially lighter in color, even after a 24 hr. blowing treatment.

Increase in viscosity seemed closely associated with increase in amount of free acid. It has been suggested that free erucic acid is a catalyst for this type of oxidative polymerization (4). This may account for the low rate of thickening of the alkali refined oils. However, other factors besides free acid must be involved as lecithin free rapeseed oil with more free acid than the corresponding alkali refined oil thickened even more slowly. Further, flash heated rapeseed oil (F. F. A., 0.74%) increased in viscosity more rapidly than bleached crude oil (F. F. A., 1.40%). This suggests that thermal degradation of lecithin and other froth producing substances accelerated the oxidation reactions.

Oils blown for 24 hr. were miscible in a mixture of five parts ethyl acetate and from eight to 10 parts of absolute ethanol; and in a mixture of five parts ethyl acetate and about four parts denatured ethanol. The relation between alcohol solubility and viscosity was not direct, for bleached rapeseed oil (viscosity 348 S. U. sec.) tolerated about the same amount of denatured ethanol as flash heated crude rapeseed oil (viscosity 1931 S. U. sec.). The results indicated that the removal of some of the free fatty acid present in the blown oil, or esterification with glycerol or ethanol, improved the alcohol solubility. There was an indication that preblowing peroxide treatment may have slightly increased alcohol toleration of oils blown to a low viscosity.

These results demonstrate the general similarity of erucic acid oils. However, the more unsaturated weed seed oil tended to thicken more rapidly than rapeseed oil, and showed relatively better alcohol toleration for the shorter blowing periods but gave similar values after extended treatment. Nevertheless, blown weed seed oil was inferior to blown rapeseed oil in paraffin miscibility. Consequently, Canadian erucic acid oils destined for industrial utilization should be segregated and not mixed prior to sale. While existing commercial specifications (1, 8) are sufficiently exact to differentiate between rapeseed oil and mustard oils, they could permit blending a low iodine value rapeseed oil with some higher iodine value oils and yet meet the 98–106 iodine value limitation. Such admixture could be expected to reduce the value of the resulting oil for formulation of lubricants.

## Acknowledgments

The authors acknowledge helpful suggestions during the planning of the investigation and are especially grateful to J. M. Allan, Canadian Oil Companies Limited, F. E. Brown, Yocum Faust Limited, and R. C. Hawkins, Canadian Industries Limited. The technical assistance of R. Cyr is acknowledged.

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## Appendix

TABLE I

MISCIBILITY OF BLOWN OILS WITH MINERAL OIL

| Oil | Number* | Blend, gm. No. 5 red paraffin oil to gm. blown oil | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 90: 10 | | 85: 15 | | 80: 20 | | 95: 5 | |
| | | Temperature of observation, °F. | | | | | | | |
| | | 75 | 35 | 75 | 35 | 75 | 35 | 75 | 35 |
| **Blown 6 hr.** | | | | | | | | | |
| Weed seed | 1 | C | C | C | C | C | C | C | C |
| Weed seed | 2 | C | H | C | H | C | H | C | H |
| Weed seed | 3 | C | C | C | C | C | C | C | H |
| Rapeseed | 4 | C | H | C | H | C | H | C | H |
| Rapeseed | 5 | C | C | C | H | C | H | C | H |
| Rapeseed | 7 | C | C | C | C | C | C | C | C |
| Rapeseed | 8 | C | H | C | H | C | H | C | H |
| Mustard seed | 9 | C | C | C | C | C | C | C | C |
| **Blown 12 hr.** | | | | | | | | | |
| Weed seed | 1 | C | H | C | H | C | H | H | H |
| Weed seed | 2 | C | H | C | H | C | H | H | S |
| Weed seed | 3 | C | C | C | H | C | H | C | H |
| Rapeseed | 4 | C | H | C | H | C | H | C | H |
| Rapeseed | 5 | C | C | C | H | C | C | C | H |
| Rapeseed | 7 | C | C | C | C | C | C | C | C |
| Rapeseed | 8 | C | C | C | C | C | C | C | C |
| Mustard seed | 9 | C | C | C | C | C | C | C | C |
| **Blown 18 hr.** | | | | | | | | | |
| Weed seed | 1 | C | H | C | H | C | H | H | S |
| Weed seed | 2 | H | S | H | S | H | S | H | S |
| Weed seed | 3 | H | S | H | S | C | H | H | S |
| Rapeseed | 4 | C | H | C | H | C | H | C | H |
| Rapeseed | 5 | C | C | C | C | C | C | C | C |
| Rapeseed | 7 | C | C | C | C | C | C | C | C |
| Rapeseed | 8 | C | H | C | H | C | H | C | H |
| Mustard seed | 9 | C | H | C | H | C | H | H | S |
| **Blown 24 hr.** | | | | | | | | | |
| Weed seed | 1 | H | S | H | S | H | S | H | S |
| Weed seed | 2 | H | S | H | S | H | S | H | S |
| Weed seed | 3 | H | S | H | S | H | S | H | S |
| Rapeseed | 4 | C | H | C | H | C | H | H | H |
| Rapeseed | 5 | C | C | C | C | C | C | C | C |
| Rapeseed | 7 | C | H | C | H | C | H | C | H |
| Rapeseed | 8 | H | S | H | S | H | S | H | S |
| Mustard seed | 9 | C | S | C | H | C | H | H | S |
| **Blown 36 hr.** | | | | | | | | | |
| Rapeseed | 7 | S | S | S | S | S | S | H | H |
| Rapeseed | 8 | S | S | S | S | S | S | H | H |
| Commercially available blown rapeseed oil, viscosity 850 S.U. sec. | | C | H | C | H | C | H | H | H |

*NOTE: C, H, and S represent condition of blend, respectively, as clear, hazy, or separated.*

* *Numbers represent oils designated in Table I.*

# A SHEET SPECIMEN SCANNER FOR X-RAY DIFFRACTION[1]

By D. J. Neil[2]

## Abstract

An improved and simple design for an X-ray diffraction scanner is described. Simultaneous back reflection and transmission photographs at distances of 3 and 5 cm. respectively and at any glancing angle from 0° to 90° are possible. The area scanned is approximately 2 cm.² at rates of either 2 sq. cm. per hour or 4 sq. cm. per hour. Useful exposures can be obtained with exposure times as low as 10 min.

The X-ray diffraction method offers a satisfactory solution to many problems involving the determination of the degree and type of preferred orientation existing in metallic sheet specimens. From an appropriate series of photograms one can obtain data necessary to plot the well known pole figure. In many cases, however, the grain size of polycrystalline specimens will produce a spotty and uneven pattern unless the X-ray beam is made to scan a definite area of the sheet sample. Such scanning action may also be necessary when one uses the back reflection technique to detect lattice parameter changes after solution heat treatment.

Various designs for mechanisms by means of which such scanning action could be secured have been proposed (1, 2, 3, 4). One disadvantage of some of these devices has been their mechanical complexity. Hay's original design (2), developed in these Laboratories, was aimed at simplicity and had many good features, one being the method for imparting the horizontal oscillatory motion to the sample. The same mechanism has been used on our new model. The original scanner had limitations, however, one being the impossibility of making exposures with glancing angles less than about 80°. Hickman and Kleinknecht (3) of the Westinghouse Research Laboratories, East Pittsburgh, Pa., have built an instrument which also enables one to take back-reflection and transmission photographs simultaneously. The oscillatory motion is obtained by using limit switches and the vertical motion by using a pawl and teeth cut in a vertical feed column. The sample holder permits rotation of the sample about a horizontal axis and the movable horizontal plate permits rotation about a vertical axis. The sample holder frame—necessitated by the rotation of the sample about a horizontal axis—obscures the beam for glancing angles less than 30°. Thewlis and Pollock (4) of the Atomic Energy Research Establishment, Harwell, Berks, England, have described a design which has all the properties of the Westinghouse instrument mentioned above with the added advantage that the scanning area can be changed by replacing cams in the mechanical driving mechanism. This scanner is, however, considerably more complicated in appearance than either the Hay or the Hickman models.

[1] *Manuscript received September 12, 1950.*

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*Contribution from the Physics Division, Aluminium Laboratories Limited, Kingston, Ontario.*

The latest model developed at these Laboratories is shown in Fig. 1. With this instrument one is able to obtain back-reflection and transmission photographs simultaneously. The minimum sample to film distances are 3 and 5 cm.

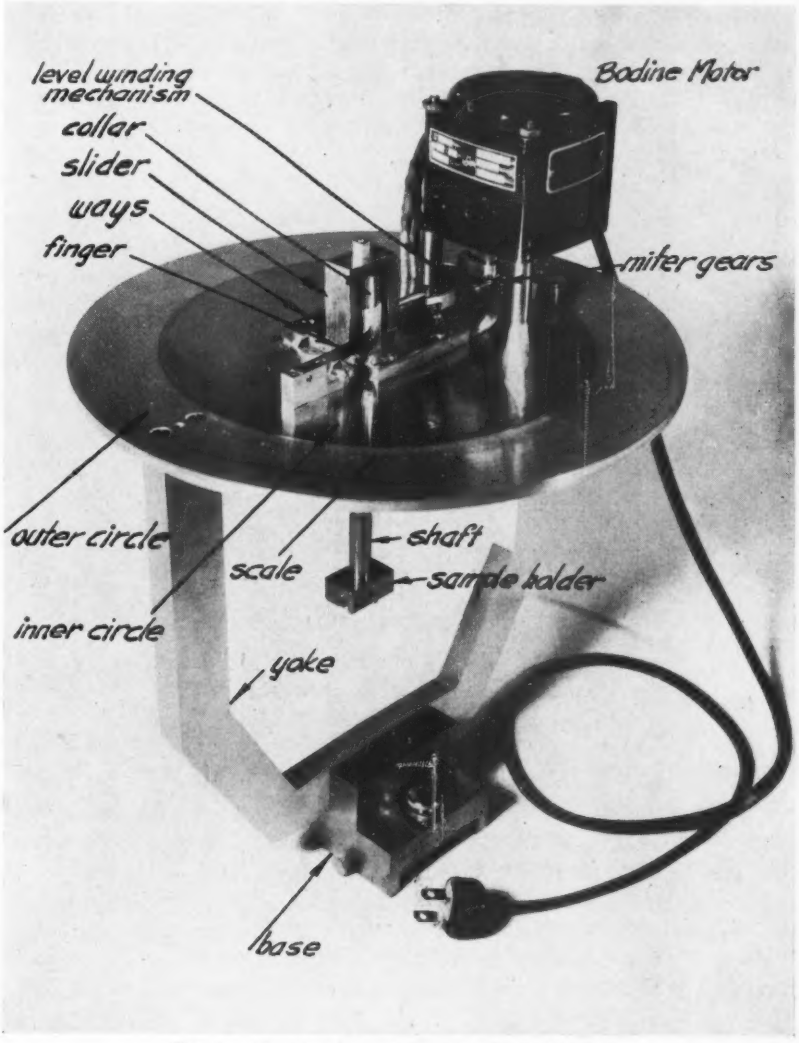


FIG. 1. *Sheet specimen scanner for X-ray diffraction.*

respectively. The glancing angle may be set at any value from 0° to 90° and the film to specimen distance remains constant during the exposure. The periodic horizontal motion is effected by a level winding mechanism from an

ordinary fishing reel which is driven through miter gears by a Bodine No. KYC-23 gear-reducer synchronous motor. The vertical motion is imparted in steps by the elevation of the sample holder shaft which extends through a collar in the slider. The shaft possesses a threaded section which moves inside the threaded collar. Teeth cut on the outside of this collar are engaged by a spring steel finger which rotates the collar at the end of each stroke. The rotation of the vertically fixed collar raises the sample an appropriate distance. By adjusting the position of the spring steel finger one can double the scanning rate. This is done by rotating the collar two teeth at a time instead of one. The elevating mechanism is shown in Fig. 2.

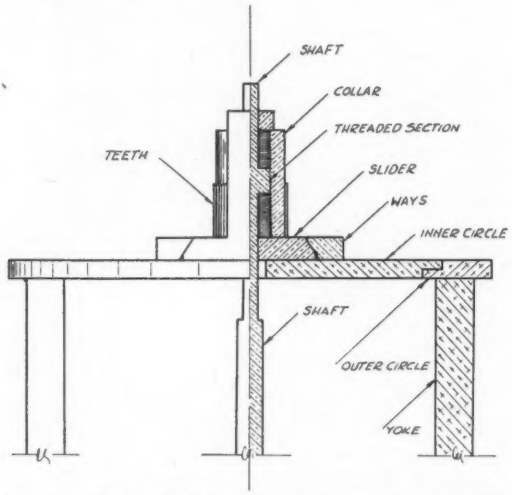


FIG. 2. *Section sketch showing sample elevating mechanism.*

The normal scanning is approximately 2 sq. cm. per hour which can be doubled to 4 sq. cm. per hour.

Both the outer circle and its supporting yoke were made of aluminum and the center circle and other parts of the mechanism were machined from brass. The base was made to fit the track of the General Electric XRD unit.

The operation of this type of scanner is mechanically simple and it has the advantage over the earlier model of requiring only one motor. One could contrive various other methods for rotation of the collar in the elevation of the specimen but the one described above has operated very satisfactorily.

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# PRODUCTION AND PROPERTIES OF 2, 3-BUTANEDIOL

## XXXIV. SOME FACTORS AFFECTING THE FERMENTATION OF BEET MOLASSES BY *BACILLUS POLYMYXA*[1]

By F. J. Simpson[2] and D. W. Stranks[2]


### Abstract

Strains of *Bacillus polymyxa*, selected for their ability to ferment glucose to butanediol, were improved in their ability to ferment beet molasses by: initial acclimatization of the organisms to beet molasses; adjusting the pH of the medium to 5.6 prior to sterilization, since this yielded the optimum pH of 6.2 after autoclaving; and by the addition of such organic substances as yeast extract, wheat bran, etc. Fermentation of 10% molasses containing 0.11% of wheat bran at 35°C., with aeration or agitation, was complete in 60 hr. and yielded 2.04 gm. diol and 0.88 gm. ethanol per 100 ml. of medium.


### Introduction

2, 3-Butanediol has been successfully produced by fermentation with *Bacillus polymyxa* from whole wheat (4), starch (5), and barley (15). These raw materials, however, are comparatively expensive and have been estimated to account for almost half the cost of producing diol on an industrial scale. The object of the present study was to obtain preliminary information on the use in this fermentation of beet molasses, a cheaper carbohydrate source than any of the cereals.

Little has been published on the use of molasses for 2, 3-butanediol fermentation. A process was patented in 1933 by Kluyver and Scheffer (7) for the fermentation of beet molasses by *Aerobacter aerogenes*, claiming a diol yield of 30%–50% of the sugar fermented. Addition to the medium of small amounts of superphosphate and of urea or an inorganic nitrogen source was considered essential. In 1944, a cane molasses fermentation process was described by Torres and Frias (16). A yield of 4% diol, 0.55% acetylmethylcarbinol (acetoin) and 0.45% ethanol was obtained from the fermented mash. In 1947, Freeman and Morrison (6) reported a conversion to diol of 36%–39% of the sugar in blackstrap molasses by *A. aerogenes*. Their molasses medium was fortified with potassium monohydrogen phosphate, ammonium sulphate, magnesium sulphate, potassium chloride, and ferric sulphate, with calcium carbonate added as a buffering agent. To the best of our knowledge, no studies have been previously published on diol production from beet molasses by *B. polymyxa*.

[1] *Manuscript received October 6, 1950.*
*Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 101 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2311.*
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## Materials and Methods

Glucose fermentation trials were made on 174 strains of *B. polymyxa* from the culture collection of the Division of Applied Biology. The test medium consisted of 10% glucose, 1% yeast extract, and 2% calcium carbonate. The 10 most active strains were then acclimatized to molasses by serial transfer, over a period of three months, through a 0.05% yeast extract – 1% calcium carbonate medium in which the molasses content was progressively increased from 3% to 16%. Compared to the original strains, these acclimatized strains gave increases in diol yield of from 0.12 to 0.86 gm. per 100 ml. of molasses medium. Further acclimatization to different samples and concentrations of molasses caused no significant improvement. Stock cultures of the strains were stored at 4°C. on a 2% agar medium containing 5% molasses, 0.5% yeast extract, and 0.05% calcium monophosphate. Prior to inoculation in a test medium, transfers were made from fresh slants to a molasses broth of the same composition.

The substrate used in this study was mainly a beet molasses of the 1946 crop, obtained from the Canada and Dominion Sugar Co. Ltd., Chatham, Ont. Some results with this molasses were checked against other samples: Chatham 1947, Manitoba 1947, Alberta 1947, and Alberta "Stefan-processed" 1947 molasses. The standard medium initially employed consisted of 10% molasses, 0.05% yeast extract, and 1% calcium carbonate. Subsequent changes in its composition are described in later sections.

Two to five milliliters of a 24 hr. culture of the organism on molasses broth was used as inoculum. In early trials the medium was incubated at 32°C. without shaking. Later, a temperature of 35°C. and shaking, by a Gump rotary shaker, were employed. Details of individual trials are given in a later section.

The procedures used for diol and ethanol analysis have been described in a previous paper (13). No correction was made for acetoin in the diol estimation. The colorimetric method of Sumner, as modified by Kolmer and Boerner (8, 14) was used for routine sugar determinations and the Lane–Eynon method (12) when a more accurate method was required.

## Experimental

The factors investigated fall into two main groups—those involving treatment of the molasses and those involving the conditions of fermentation.

### Effect of Molasses Treatment on Diol Yield

Factors involving treatment of the molasses included the effects on diol yield of different sterilization procedures, of neutralization procedures, of steam sterilization on the pH, and of clarification procedures.

The effects on diol yield of various sterilization procedures, applied to 10% molasses solutions adjusted to pH 6 with sulphuric acid, are summarized in Table I. Two different strains of *B. polymyxa* were used in this test and the

yields of diol and ethanol were determined after 72 hr. The results show that heat treatment at 15 p.s.i. gauge pressure for 15 min. gave the best results. Prolonged heat treatment was detrimental. The increased yields obtained by vacuum filtration and steam sterilization could probably be attributed to the removal of volatile toxic substances (17). As a result of this trial a 15 min. sterilization period at 15 p.s.i. gauge pressure was adopted as standard procedure.

TABLE I

THE EFFECT OF STERILIZATION PROCEDURES ON THE YIELD OF TOTAL PRODUCTS (DIOL AND ETHANOL)

| Treatment | Total products, gm./100 ml. | |
|---|---|---|
| | Strain C2(1) | Strain C3(2) |
| Filtered under pressure | 1.50 | 1.48 |
| Filtered under vacuum | 1.76 | 1.84 |
| Boiled for 20 min., made up to the original volume, and filtered under pressure | 1.70 | 1.78 |
| Autoclaved at 15 p.s.i. gauge pressure for 15 min. | 1.86 | 1.95 |
| Autoclaved for 30 min. at 15 p.s.i. gauge pressure | 1.79 | 1.84 |
| Autoclaved for 60 min. at 15 p.s.i. gauge pressure | 1.49 | 1.60 |
| Autoclaved for 180 min. at 15 p.s.i. gauge pressure | 1.13 | 1.24 |

Molasses solutions have a pH of about 9 and for maximum diol yield must be adjusted to the optimal pH of the test organism. With *B. polymyxa* this is approximately pH 6.2. Solutions containing 10% molasses were adjusted to pH 6 with various acids, and sterilized. Sterile calcium carbonate was then added to alternate sets of media as a buffer, and they were fermented at 35°C. for 72 hr. with agitation (Table II). Formic, acetic, and hydrochloric acids were the most effective for diol production. When calcium carbonate was added an objectionable slime was formed and lower yields were obtained, except with phosphoric acid. For subsequent work, except when otherwise stated, acetic

TABLE II

EFFECT ON DIOL YIELD OF NEUTRALIZING WITH VARIOUS ACIDS BEFORE STERILIZATION

(Averages of three fermentations)

| Acid | Without calcium carbonate as buffer | | With 1% calcium carbonate as buffer | |
|---|---|---|---|---|
| | Final pH | Diol, gm./100 ml. | Final pH | Diol, gm./100 ml. |
| None | 5.29 | 0.55 | – | – |
| Formic | 5.67 | 1.77 | 6.80 | 1.55 |
| Acetic | 5.76 | 1.73 | 6.75 | 1.65 |
| Sulphuric | 5.41 | 1.57 | 6.80 | 1.60 |
| Phosphoric | 5.31 | 1.39 | 5.94 | 1.86 |
| Hydrochloric | 5.46 | 1.71 | 6.66 | 1.64 |
| Nitric | 5.67 | 1.64 | 6.75 | 1.47 |

acid was used to adjust the pH before fermentation. The beneficial effects from the addition of this acid to diol fermentation substrates have been described by Roberts (10).

Some preliminary experiments were carried out on adjustment of the pH of the molasses to different levels before sterilization. The pH always rose during sterilization. An initial pH of 5.3–5.8 was found to be the most satisfactory as the pH subsequently rose during sterilization to 6.3, approximately the optimum for the butanediol fermentation with *B. polymyxa*. No readjustment of the pH after sterilization was therefore necessary. This procedure was adopted as standard for further work. Experiments in which the medium was sterilized at a lower pH than 5.5 showed that slightly higher yields of diol might be obtained but this procedure has the disadvantage that it requires readjustment of the pH of the medium before fermentation.

Some of the commercial methods of clarifying molasses were tested and the diol yields obtained on such treated media were compared with those obtained with untreated media. Precipitation with carbonate (2), superphosphate (17), "Calgon" (sodium hexametaphosphate) and sodium phosphate, zinc hydroxide, tannic acid, tannic acid and lime, and potassium ferrocyanide and oxidation with hydrogen peroxide caused no improvement.

## Effect of Fermentation Conditions on Diol Yield

Factors involving the conditions of fermentation included the effects of the size of inoculum, of molasses concentration, of aeration, agitation, and surface–depth ratios, of antifoaming agents, of incubation temperature, and of nutrients added to the medium.

### *Effect of the Amount of Inoculum on the Fermentation*

As already described, the composition of the inoculum medium differed slightly from that of the fermentation medium. After 24 hr. incubation in this medium, a direct count with a Petroff–Hauser plate showed a population of 1200 million organisms per milliliter. This figure compared favorably with that obtained by Ledingham *et al.* (9) and by Blackwood and Ledingham (3) who used a starch – yeast-extract inoculum medium for the fermentation of wheat.

TABLE III

Effect of the amount of inoculum on diol formation

| Inoculum, % by volume | Diol, gm./100 ml. | |
|---|---|---|
| | 48 hr. | 72 hr. |
| 0.01 | 0.57 | 1.94 |
| 0.05 | 0.97 | 1.89 |
| 0.10 | 1.14 | 1.86 |
| 0.50 | 1.42 | 1.72 |
| 1.00 | 1.42 | 1.69 |
| 5.00 | 1.40 | 1.83 |

Inocula, added to the ordinary fermentation medium in amounts varying from 0.01 to 5.0% by volume, gave diol yields after 48 and 72 hr. incubation as shown in Table III. These results show that increasing amounts of inoculum up to 0.5% accelerated the fermentation, but that after 72 hr. the highest yields were obtained in those flasks which had received the smallest amounts of inoculum. Therefore it appears that, in the fermentation of beet molasses, large amounts of inoculum are unnecessary.

*Effect of the Concentration of Molasses on the Diol Yields*

Solutions containing 10%, 12.5%, 15%, 17.5%, and 20% molasses, supplemented with 0.11% wheat bran, were inoculated and incubated as usual for six days. Duplicates were withdrawn every 24 hr. and analyzed for residual sugar, diol, and ethanol. The yields of diol + ethanol are plotted in Fig. 1.

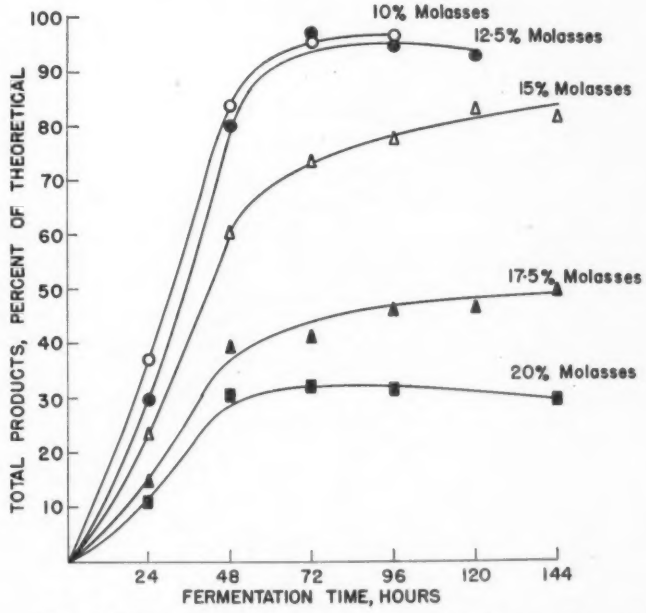


FIG. 1. *Effects of different concentrations of molasses in the medium on the formation of "total products" (diol + ethanol).*

They are expressed as percentages of what is assumed to be the theoretical yield, namely 50% conversion of sugar to "total products" (diol + ethanol). The curves show that a molasses concentration of 10% was the best concentration tested. With this concentration the fermentation is finished in about 72 hr. Fermentation of higher concentrations was slower and sometimes was not complete in six days.

*Effects of the Surface–Depth Ratio and of Agitation and Aeration*

In the earlier experiments unshaken cultures were used to investigate the effect of the surface–depth ratio on the rate of fermentation. Aliquots of 300 ml. of medium were fermented in Pyrex reagent bottles of various volumes ranging from 500 ml. to nine liters. Residual sugar was determined after 48 and 72 hr. and the results are shown in Table IV. Up to 48 hr. the effect of the ratio was

TABLE IV
EFFECT OF SURFACE DEPTH RATIO ON SUGAR DISSIMILATION

| Surface/depth ratio | Sugar dissimilated, % | |
|---|---|---|
| | 48 hr. | 72 hr. |
| 300 | 38 | 78 |
| 100 | 32 | 72 |
| 40 | 35 | 42 |
| 15 | 28 | 40 |
| 5 | 28 | 32 |

only slight, but after that time, improvement of the fermentation by the higher surface–depth ratios became more apparent. These results suggested that aeration (or both aeration and agitation) was necessary.

In the next experiments the effect of agitation and aeration was studied. Aliquots of 300 ml. of medium were fermented in one liter Erlenmeyer flasks, with and without agitation at 100 r.p.m. on a shaker. Some of the shaken flasks were also aerated at the rate of two liters per minute. Under the conditions employed (Table V) agitation apparently reduced the fermentation time considerably. The method of aeration must have been rather ineffective since it caused no improvement over flasks merely agitated.

TABLE V
EFFECTS OF AERATION AND AGITATION ON THE FORMATION OF DIOL

| Conditions | Diol formed, gm./100 ml. | | | |
|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | 96 hr. |
| Stationary | 0.31 | 0.86 | 1.56 | 1.90 |
| Agitated | 0.76 | 1.52 | 1.83 | 1.84 |
| Agitated and aerated | 0.74 | 1.49 | 1.96 | |

A similar experiment was conducted using six-liter aliquots of medium in nine-liter reagent bottles. The agitation of these deep cultures on the shaker was much less effective than that obtained in smaller flasks. Aeration however was very vigorous with three liters of air per minute passing through "Aloxite" aerators set deep in the medium. It is not surprising, therefore, that in this experiment aeration as well as agitation improved the fermentation markedly (Table VI).

TABLE VI

EFFECT OF AIR AND AGITATION ON THE FORMATION OF DIOL IN NINE LITER BOTTLES WITH SIX LITERS OF MEDIUM

| Conditions | Diol formed, gm./100 ml. | | | | |
|---|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | 96 hr. | 120 hr. |
| Stationary | 0.17 | 0.72 | 1.09 | 1.30 | 1.30 |
| Agitated | 0.40 | 1.43 | 1.87 | 1.97 | – |
| Agitated and aerated | 0.36 | 1.90 | 1.97 | – | – |

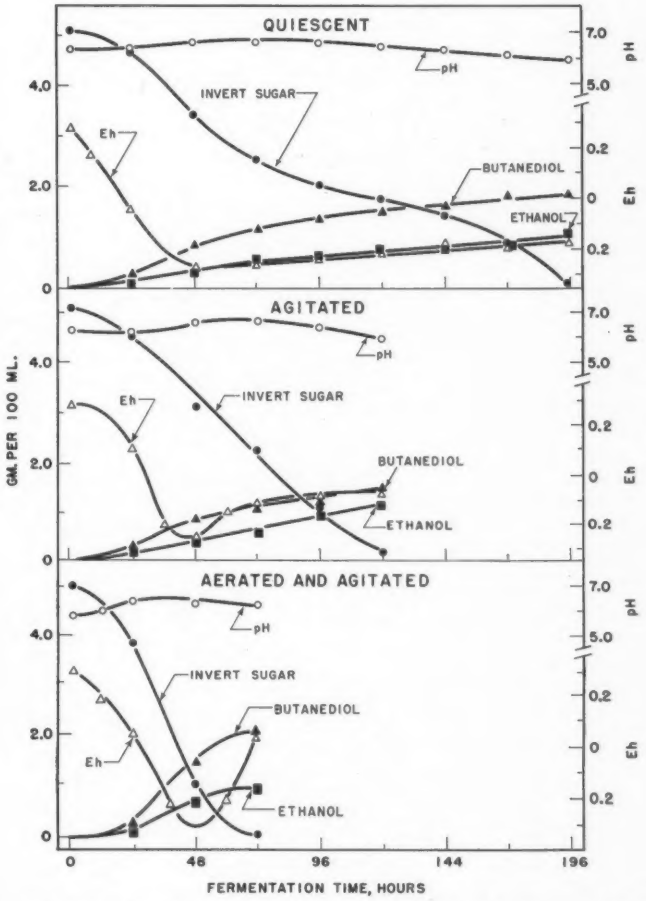


FIG. 2. *Effects of different aeration conditions on fermentation of molasses in 10-liter batches of medium.*

These results were checked on a still larger scale in 12-liter Florence flasks. The medium was agitated by a laboratory stirrer. For the aeration, sterile air, metered by a rotameter, was passed at the rate of one liter per minute through a sintered glass aerator set deep in the medium. The assembled fermentor with the medium was autoclaved for 30 min. at 15 p.s.i. gauge pressure. The medium used consisted of 10% molasses with 0.13% bran. Corn oil was added to reduce foaming. Samples were withdrawn from the flask every 24 hr. and the course of the fermentation was followed by determining diol, ethanol, residual sugar, pH, and Eh. The data in Fig. 2 showed that agitation and aeration were both essential since quiescent conditions required eight days for completion. Agitation reduced the fermentation time to four days and with agitation and aeration only three days were needed for a complete fermentation. From these experiments it seems likely that agitation and aeration would both be necessary on the pilot-plant and industrial scales. However, if either the agitator or the aerator is efficient enough, only one or the other may be necessary.

*Effect of Antifoam Agents on the Fermentation*

For deep culture fermentations, employing agitation and aeration, an effective nontoxic antifoam agent was necessary. Various commercial preparations and natural oils were compared for their ability to prevent foaming in fermentations aerated under standard conditions. All the agents listed in Table VII were found to be effective foam breakers. Their toxicity was tested in ordinary shake cultures. The agent to be tested was sterilized separately and added aseptically to the fermentation flasks. The diol yields obtained after 72 hr. incubation are set out in Table VII. The natural oils tested (corn, linseed, soyabean, and lard) and the purified derivatives such as triolein or sodium oleate were not toxic or

TABLE VII

EFFECT OF TOXICITY OF ANTIFOAM AGENTS ON DIOL PRODUCTION
(0.5 ml. agent added to 200 ml. medium in 500 ml. Erlenmeyer flask)

| Antifoam agent | Diol, gm./100 ml. |
|---|---|
| Control | 2.04 |
| Lard oil | 2.10 |
| Corn oil | 2.00 |
| Linseed oil | 2.00 |
| Triolein | 1.99 |
| Soyabean oil | 1.99 |
| 3% octadecanol in lard oil | 1.95 |
| Sodium oleate | 1.93 |
| Antifoam A* | 1.88 |
| Vegifat Y** | 1.55 |
| Turkey Red oil | 0.63 |
| C-10 antifoam*** | 0.11 |
| L.F. antifoam*** | 0.08 |
| H.F. antifoam*** | 0.06 |

*Dow Corning Co., Midland, Mich.
**Nopco Chemical Co., Specialties Sales Division, Harrison, N.J.
***I. DuPont de Nemours & Co. (Inc.), Ammonia Department, Wilmington, Del.

else very slightly so. The synthetic antifoam agents all seemed to inhibit the fermentation in varying degrees.

*Effect of Incubation Temperature on the Fermentation*

Flasks were incubated at a series of temperatures ranging from 30° to 40°C. Two strains of *B. polymyxa* were used in this test and the products were analyzed after 48, 72, and 96 hr. The results in Table VIII show that a temperature of 36°C. or slightly higher was the optimum for this fermentation.

TABLE VIII

EFFECT OF TEMPERATURE ON THE PRODUCTION OF DIOL

| Strain | Temperature, °C. | Diol, gm./100 ml. | | |
|---|---|---|---|---|
| | | 48 hr. | 72 hr. | 96 hr. |
| C 3(2) | 30 | 0.64 | 1.33 | 1.60 |
| | 32.5 | 1.03 | 1.63 | 1.58 |
| | 35 | 1.04 | 1.76 | 1.82 |
| | 37.5 | 1.11 | 1.17 | 1.52 |
| | 40.0 | 0.99 | 0.99 | 1.33 |
| C 2(1) | 30 | 0.78 | 1.24 | 1.52 |
| | 32.5 | 0.78 | 1.14 | 1.73 |
| | 35 | 1.03 | 1.43 | 1.96 |
| | 37.5 | 1.29 | 1.43 | 2.01 |
| | 40.0 | 1.04 | 1.12 | 1.47 |

*Requirements for Additional Nutrients*

As mentioned earlier, Kluyver and Scheffer (7) and Freeman and Morrison (6), but not Torres and Frias (16), found it essential to add mineral supplements to molasses. These divergent findings made it highly desirable to test the molasses media used in this investigation for possible mineral deficiencies. Simultaneously, the influence of added organic substances was tested.

The procedure adopted was to test singly the effect of a number of adjuvants. A basal medium fortified with those found to be most beneficial was then devised. From this complete medium each of the substances was omitted in turn, or two or more of them simultaneously. In this way the medium was gradually simplified until only those substances responsible for significant stimulation of the fermentation were retained in the formula.

The mineral substances added were: ferric hydroxide, potassium permanganate, calcium monophosphate, magnesium sulphate, and various nitrogen compounds. None of the inorganic nitrogen-containing compounds had a beneficial effect, suggesting that the molasses had adequate available nitrogen.

The organic stimulants tried were: yeast extract, malt sprouts, ground whole wheat, wheat bran, ground whole barley, straw, ground whole corn, and wheat germ. These appeared to be more stimulating than the inorganic compounds. The effect of these organic materials on the dissimilation of sugar is shown in Table IX, in which the substances under test were added to a 10% molasses

TABLE IX

EFFECT OF ORGANIC SUBSTANCES ON THE SUGAR DISSIMILATION

| Substance added 0.10 gm./100 ml. | Invert sugar dissimilated, gm./100 ml. | |
|---|---|---|
| | 48 hr. | 72 hr. |
| Control | 1.0 | 3.3 |
| Yeast extract | 4.1 | 4.6 |
| Malt sprouts | 3.9 | 4.5 |
| Whole wheat | 3.7 | 4.7 |
| Wheat bran | 2.2 | 3.8 |
| Whole barley | 1.8 | 3.8 |
| Straw | 1.7 | 2.6 |
| Whole corn | 1.6 | 3.8 |
| Wheat germ | 1.6 | 3.1 |

medium (5 gm. of invert sugar per 100 ml. of medium) containing no added inorganic salts. Yeast extract, malt sprouts, whole wheat, and wheat bran were the most effective, but all the substances tested gave some increase in the rate of sugar utilization. Since some of these organic substances had an equally marked effect on the fermentation, wheat bran, the cheapest of the more efficient adjuvants, was adopted as a constant constituent of the medium.

To determine the optimum concentration of bran, concentrations of from 0.03 to 0.15 gm. per 100 ml. of medium were tested. The phosphate concentration was kept constant at 0.035 gm. per 100 ml. The results for 72-hr. fermentations are shown in Table X. A concentration of 0.11 to 0.12 gm. of bran per 100 ml. of medium was sufficient to ensure maximal yield of diol.

TABLE X

EFFECT OF BRAN CONCENTRATION ON THE PRODUCTION OF DIOL

| Bran, gm./100 ml. | Diol, gm./100 ml.* |
|---|---|
| None | 1.75 |
| 0.03 | 1.78 |
| 0.06 | 1.89 |
| 0.09 | 1.96 |
| 0.12 | 1.98 |
| 0.15 | 1.89 |

* *Average of six separate batches of molasses solutions. Standard deviation = .039 for individual determinations.*

It was found that all the inorganic compounds mentioned previously could then be eliminated from the medium, with the exception of phosphate. For the molasses medium with the optimum concentration of bran a slight increase in diol yield in 72 hr. was caused by the addition of phosphate (Table XI). As the increase was not high enough to be economically significant, phosphate was also eliminated from the medium.

TABLE XI

EFFECT OF MONOCALCIUM PHOSPHATE ON THE PRODUCTION OF DIOL

| Phosphate, gm./100 ml. | Diol, gm./100 ml.* |
|---|---|
| None | 2.10 |
| 0.005 | 2.08 |
| 0.01 | 2.10 |
| 0.02 | 2.11 |
| 0.04 | 2.11 |
| 0.08 | 2.14 |

* *Averages of six separate batches of molasses solutions. Standard deviation = .039 for individual determinations.*

As a result of the foregoing work, the formula of the medium was simplified to 10% molasses with 0.11% bran, acidified before sterilization with acetic acid to pH 5.6. This result was obtained using media prepared from Chatham molasses of the 1946 crop. It was checked with other samples of molasses and considerable differences were found. Some required no added bran while others required 0.20 gm. per 100 ml.

## Acknowledgments

The authors wish to extend sincere thanks to Dr. Hopkins and staff for outlining and calculating the statistical work, and to Dr. D. Murphy for valuable assistance at all times.

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# THE BRIQUETTING OF STRAW[1]

By J. E. Stone


## Abstract

Small discus-shaped briquettes weighing approximately 15 gm. each have been made from wheat straw by the application of heat and pressure without the use of a binder. The most suitable temperature is considered to be 220°C., the size of hammered straw 2 in., and a minimum of 5000 lb./sq. in. for the pressure. Higher pressures produce a stronger and more moisture-resistant product and enable the pressing time to be reduced and the thickness of the briquette to be increased. A moisture content in the raw material of less than 10% is recommended. The efficiency of the briquetting process has been determined for certain idealized conditions and it has been found that about 50 times as much energy may be obtained from the briquettes by burning as is required for their manufacture. Further information, particularly of an economic nature, can only be obtained with a full scale machine running at its normal operating speed as in this way the important effect of frictional heating can be determined.


## Introduction

The large tonnage of straw annually produced on the Prairies has for many years received attention as a possible source of fuel, but the same obstacle is met with as in other contemplated outlets for this material, namely, the problem of collection. There are few locations where straw is concentrated to a greater extent than several hundred tons and for this reason a light, portable briquetting machine which could be driven to the strawstacks, or used in conjunction with a pick-up baler, would perhaps be the most suitable method of getting around the collection difficulty. Such a machine is not available commercially and a survey of the literature did not yield much information which would lead to the design of one.

There is a wealth of information on which to draw when considering briquetting in general, owing to the long established practice of briquetting low-grade coals and slack, and the U.S. Department of the Interior has recently put out a bibliography on the subject containing over 300 references (7). In the main, the data on coal cannot be applied to straw because (*a*) coal briquetting machinery is designed to be stationary and is massive in construction, and (*b*) coal dust requires less compacting than straw and the small volume change of the former material on compression allows certain types of molding techniques not directly applicable to straw. With sawdust the problem has been to dispose of the large stockpiles accumulating at the sawmills and, as with coal, little consideration has been given to the design of a portable machine. The literature describes a number of sawdust briquetting processes, varying greatly in complexity, some using inorganic (6, 10, 17) or organic (3, 5, 8, 11, 12, 13, 14, 20,

[1] *Manuscript received July 11, 1950.*
*Contribution from the Prairie Regional Laboratory, National Research Council, Saskatoon, Sask. Issued as Paper No. 98 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2310.*

21, 23) materials as binders, while others rely only on the effect of heat and pressure (2, 4, 18, 25). The same is true of briquetting other vegetable wastes such as straw, sunflower seed hulls, bagasse, etc. (9, 16, 19, 24), elaborate pretreatments often being described. It is noteworthy that the only process which has achieved commercial success, i.e., the "Pres-to-log", uses the operationally simple principle of high pressures and the attendant heating effect of friction. The "Pres-to-log" process has been applied to a great many vegetable wastes and it appears that they are all equally adaptable to this method of briquetting, producing a strong, dense product with good burning characteristics (22), but owing to the size of the machine it has only been used commercially for sawdust and other wastes where there is a large and constant supply of raw material. One of the chief factors governing the size of the machine is the load which is imposed by the high pressures developed during briquetting. Thus the "Pres-to-log" machine has to sustain a load of 200,000 lb. at one stage in its operation and the excessive heat produced is removed by water cooling. Nevertheless, the use of high pressures alone is attractive and appears to offer few complications from pretreatments, mixing of binders, etc., which must be cut to a minimum in a portable machine. The fact that the "Pres-to-log" process uses very high pressures and must be water cooled leads to the possibility that somewhat lower pressures might develop just the optimum amount of heat while still producing a satisfactory product, or, alternatively, that lower pressures could be used in conjunction with heat supplied from an external source. To answer these questions it is first necessary to know the minimum pressure which will make a satisfactory briquette from straw and also the effect of temperature on this pressure. The present work was undertaken to gain some information on these points. Some consideration has also been given to the time and energy required to make a briquette since a knowledge of these factors is important economically.

## Equipment

The size and shape of a briquette will almost certainly affect its properties, and the design chosen was discus-shaped with a diameter of 1.596 in. This type is somewhat similar to the pillow-shaped briquettes on the market and has the advantage that it can be readily made in a piston and cylinder type mold with concave upper and lower surfaces. With such curved surfaces they should be easy to handle with a shovel and their size would permit feeding in through an automatic stoker. The diameter of 1.596 in. gives a cross section area of 2 sq. in. A diagram of the mold is given in Fig. 1. It was provided with gas vents in the upper and lower surfaces and also in the sides so that gas could escape from the briquette. The intermittent nature of laboratory operations ruled out the possibility of heating the straw by friction so the whole mold was electrically heated and the temperature controlled by varying the voltage with a Variac. The temperature was measured with a thermocouple inserted in the periphery of the mold so that the tip would come as close as possible to the surface of the briquette.

A hand-operated hydraulic press with electrically heated platens and a capacity of 20 tons was used for this work. The maximum pressure obtainable was therefore 20,000 lb./sq. in.

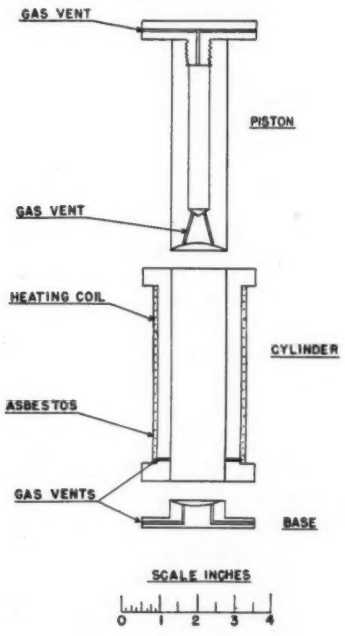


FIG. 1. *Briquetting mold.*

For testing the ability of the briquettes to withstand rough-handling the A.S.T.M. Small Jar Tumbler Test (1) used for coal was adopted. The apparatus consists of a steel cylinder 6 1/2 in. long and 6 7/8 in. in diameter on the inside of which are four projecting vanes 1 in. high. The cylinder is placed inside a laboratory ball mill and rotated at 40 r.p.m., the briquettes thus receiving many light blows in the course of a minute.

## Procedure

After introducing a weighed amount of straw into the mold the desired pressure was applied as rapidly as possible. With the equipment at hand this took approximately 10 sec. The pressure was maintained for various lengths of time up to 75 sec. but for the main part of the work 30 sec. was arbitrarily adopted. The pressure was released gradually over a period of 15 sec., this being a compromise between a sudden release which tended to explode the briquette and a desire to keep the pressing cycle as short as possible.

Twenty briquettes, weighing a total of about 300 gm., were used for each durability test. With coal products the mill is rotated for an hour and the material then graded through various screens, but the straw briquettes did not break up, instead merely wearing away, so that they were weighed before the test and after one, two, three, and five hours to find the percentage lost.

The ability of the briquettes to withstand moisture was obtained (*a*) by placing them in water and noting the time for complete disintegration, and (*b*) placing them in an atmosphere of 100% relative humidity and following the increase in weight over a period of six days.

A visual examination of the condition of the surface and the presence or absence of cracks around the edge of the briquettes proved to be of considerable value in judging their quality, hence a photographic record was kept illustrating the effects of temperature, pressure, and straw size.

## Results

*The Effect of Temperature, Pressure, and Straw Size*

Briquettes were made at 180°, 200°, and 220°C., using pressures of 2, 5, 10, and 20 thousand lb. per sq. in. and with 1/16th, 1/2, and 2 in. hammered wheat straw. Other factors were kept constant as follows:

| | |
|---|---|
| Time to reach pressure | 10 sec. |
| Time pressure maintained | 30 sec. |
| Time for release of pressure | 15 sec. |
| Amount of straw used | 15 gm. |
| Moisture content of straw | 4% |

Photographs of typical briquettes made under each set of conditions are shown in Figs. 2, 3, and 4. It is immediately apparent that increasing the pressure improves the product. Closer examination shows that an increase in temperature and in straw size is also beneficial. Thus while pressures of 2000 and 5000 lb. per sq. in. usually gave briquettes which were split or had cracks around the edge, those made at 220°C. and with 2 in. straw (Nos. 33 and 34) were more promising. But these latter were not consistently satisfactory and it was only when pressure of 10,000 lb. per sq. in. were used that a reasonable quality could be maintained.

The trend towards an improved product with increasing temperature, pressure, and straw size is indicated in Tables I, II, and III, which show the ability of the briquettes to withstand rough handling, water, and 100% relative humidity respectively. Most of the briquettes stood up very well to the rough handling test, losing less than 20% in the form of a fine dust after five hours' treatment and retaining their bulk in one piece. Those which were not considered good enough to test were either split or did so soon after the test was initiated. When it is considered that under the same conditions, coal is reduced to a fine rubble, it becomes apparent that many of the briquettes have ample mechanical strength. The water resistance of the briquettes as given in Table II

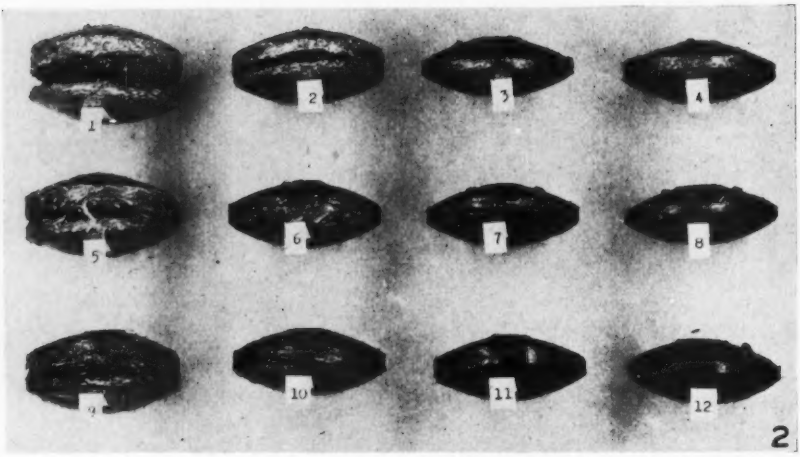


FIG. 2. *Briquettes made at 180°C.*

| *Straw size, in.* | *Pressure, lb./sq. in.* | | | |
|---|---|---|---|---|
| | *2000* | *5000* | *10,000* | *20,000* |
| | *Briquette No.* | | | |
| *1/16* | *1* | *2* | *3* | *4* |
| *1/2* | *5* | *6* | *7* | *8* |
| *2* | *9* | *10* | *11* | *12* |

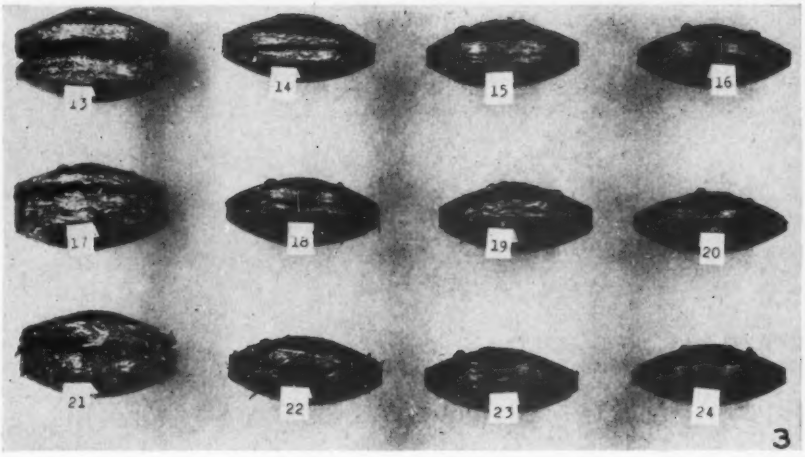


FIG. 3. *Briquettes made at 200°C.*

| *Straw size, in.* | *Pressure, lb./sq. in.* | | | |
|---|---|---|---|---|
| | *2000* | *5000* | *10,000* | *20,000* |
| | *Briquette No.* | | | |
| *1/16* | *13* | *14* | *15* | *16* |
| *1/2* | *17* | *18* | *19* | *20* |
| *2* | *21* | *22* | *23* | *24* |

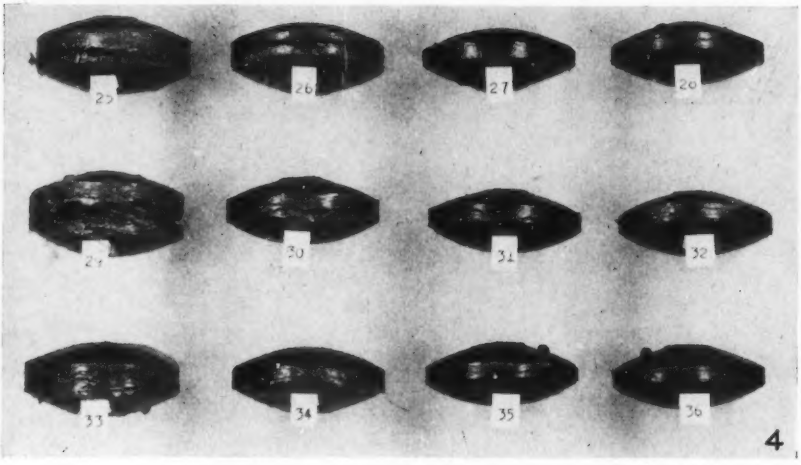


FIG. 4. *Briquettes made at 220°C.*

| *Straw size, in.* | *Pressure, lb./sq. in.* | | | |
|---|---|---|---|---|
| | *2000* | *5000* | *10,000* | *20,000* |
| | *Briquette No.* | | | |
| *1/16* | *25* | *26* | *27* | *28* |
| *1/2* | *29* | *30* | *31* | *32* |
| *2* | *33* | *34* | *35* | *36* |

TABLE I

ROUGH-HANDLING TEST

| Temperature | Pressure lb./sq. in. | 2 in. straw | | | | 1/2 in. straw | | | | 1/16 in. straw | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hours in mill | | | | | | | | | | | |
| | | 1 | 2 | 3 | 5 | 1 | 2 | 3 | 5 | 1 | 2 | 3 | 5 |
| | | % Loss | | | | | | | | | | | |
| 180°C. | 2000 | * | * | * | * | * | * | * | * | * | * | * | * |
| | 5000 | 7.1 | 12.5 | 17.3 | 23.7 | 8.8 | 15.6 | 21.1 | 32.0 | * | * | * | * |
| | 10,000 | 6.2 | 9.4 | 14.0 | 20.7 | 5.9 | 10.6 | 15.0 | 21.3 | * | * | * | * |
| | 20,000 | 4.4 | 9.1 | 14.0 | 19.6 | 4.4 | 8.1 | 11.3 | 17.0 | * | * | * | * |
| 200°C. | 2000 | * | * | * | * | * | * | * | * | * | * | * | * |
| | 5000 | 5.5 | 10.3 | 14.4 | 20.5 | 6.8 | 12.9 | 17.6 | 26.5 | * | * | * | * |
| | 10,000 | 4.9 | 9.0 | 12.7 | 18.3 | 5.5 | 9.7 | 13.7 | 19.9 | 7.0 | 11.7 | 14.9 | 20.6 |
| | 20,000 | 4.0 | 7.1 | 10.9 | 18.3 | 4.2 | 7.6 | 10.8 | 15.7 | 6.7 | 10.2 | 12.7 | 17.6 |
| 220°C. | 2000 | 5.5 | 10.2 | 13.9 | 20.0 | * | * | * | * | * | * | * | * |
| | 5000 | 5.0 | 8.9 | 12.8 | 18.6 | 6.2 | 10.2 | 14.1 | 21.4 | * | * | * | * |
| | 10,000 | 4.1 | 7.3 | 10.3 | 15.9 | 5.1 | 8.9 | 12.4 | 18.4 | 6.5 | 10.5 | 13.4 | 18.2 |
| | 20,000 | 3.5 | 6.5 | 9.1 | 14.2 | 4.1 | 7.0 | 10.0 | 14.8 | 6.5 | 10.0 | 12.3 | 16.7 |

* *The briquettes were not considered good enough to test.*

TABLE II

EFFECT OF TOTAL IMMERSION OF BRIQUETTES IN WATER AT 25°C.

| Temperature | Pressure, lb./sq. in. | 2 in. | ½ in. | 1/16 in. |
|---|---|---|---|---|
| | | Time required for total disintegration, minutes | | |
| 180°C. | 2000 | 1 | 1 | ½ |
| | 5000 | 5½ | 2½ | 1 |
| | 10,000 | 7 | 5 | 1 |
| | 20,000 | 9 | 25 | 30 |
| 200°C. | 2000 | 7 | 2½ | 1 |
| | 5000 | 8½ | 5½ | 1½ |
| | 10,000 | 100 | 6½ | 25 |
| | 20,000 | >120 | 40 | 40 |
| 220°C. | 2000 | 4½ | 1 | 1 |
| | 5000 | 10½ | 4½ | 2 |
| | 10,000 | 25 | 5 | 8 |
| | 20,000 | >120 | >120 | >120 |

is low when made at 180°C. even at high pressures, but 220°C. and 20,000 lb./sq. in. produced briquettes that did not completely disintegrate after two hours' immersion or indeed after several days. While it is to be expected that an increase in temperature and pressure would produce better briquettes, the improvement noticed on increasing the size of hammered straw from 1/16 in.

TABLE III

EFFECT OF 100% RELATIVE HUMIDITY ON BRIQUETTES, 25°C.

| Temperature | Pressure, lb./sq. in. | 2 in. straw | | | | ½ in. straw | | | | 1/16 in. straw | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hours in 100% R.H. | | | | | | | | | | | |
| | | 24 | 48 | 72 | 144 | 24 | 48 | 72 | 144 | 24 | 48 | 72 | 144 |
| | | % Gain | | | | | | | | | | | |
| 180°C. | 2000 | 4.8 | 7.3 | 9.2 | 13.9 | 7.1 | 10.8 | 12.3 | 16.4 | 7.0 | 10.2 | 12.1 | 17.0 |
| | 5000 | 4.9 | 7.4 | 9.5 | 15.0 | 5.5 | 8.2 | 10.4 | 15.5 | 6.2 | 9.1 | 11.4 | 16.8 |
| | 10 000 | 3.9 | 5.7 | 7.6 | 11.3 | 4.3 | 6.6 | 8.6 | 13.2 | 4.1 | 6.5 | 8.3 | 13.5 |
| | 20 000 | 3.6 | 5.5 | 7.2 | 10.8 | 3.2 | 5.2 | 6.7 | 11.3 | 4.2 | 6.4 | 8.2 | 13.2 |
| 200°C. | 2000 | 5.0 | 7.0 | 9.0 | 14.7 | 6.2 | 8.7 | 11.1 | 16.6 | 6.4 | 8.9 | 11.0 | 16.4 |
| | 5000 | 4.2 | 6.1 | 8.3 | 13.8 | 4.7 | 7.2 | 9.4 | 15.5 | 5.0 | 7.5 | 9.8 | 15.7 |
| | 10,000 | 3.5 | 5.0 | 6.9 | 11.2 | 3.9 | 6.0 | 8.1 | 13.0 | 2.8 | 4.5 | 7.2 | 11.2 |
| | 20,000 | 2.9 | 4.7 | 6.6 | 11.0 | 3.3 | 5.3 | 6.6 | 11.2 | 2.8 | 4.4 | 7.1 | 11.0 |
| 220°C. | 2000 | 4.5 | 7.4 | 9.8 | 14.3 | 4.6 | 7.5 | 10.2 | 15.5 | 5.9 | 9.8 | 13.3 | 20.7 |
| | 5000 | 4.1 | 7.1 | 9.2 | 13.7 | 5.7 | 9.2 | 11.6 | 16.1 | 4.6 | 8.0 | 11.1 | 17.1 |
| | 10.000 | 3.3 | 5.8 | 7.8 | 12.5 | 4.6 | 7.5 | 9.8 | 14.8 | 3.1 | 5.3 | 7.3 | 12.7 |
| | 20,000 | 3.0 | 5.4 | 7.0 | 11.0 | 3.3 | 5.5 | 7.3 | 11.5 | 3.2 | 5.7 | 8.0 | 12.8 |

to 2 in. is somewhat surprising. It would be expected that the finer material would pack more tightly and give a more cohesive product, but that the reverse is true suggests that the dominating factor is the ability of the steam and other gases to escape from the interior of the briquette on releasing the pressure. Here the time element comes in, an important factor in commercial production, and the possibility of cutting down the time was investigated.

*Minimum Time to Make a Briquette*

There are three occasions during the pressing cycle when the time might be cut down, and the first or 10 sec. period for the application of pressure could almost certainly be reduced to negligible proportions in a commercial machine. The second two are more important to the briquetting operation and require some consideration. It was found imperative to release the pressure slowly and the 15 sec. period used here could not be reduced to any great extent. The 30 sec. pressing period is susceptible to variation, however, and this period was increased or decreased until a satisfactory briquette was obtained under all conditions of temperature and pressure. The results are recorded in Table IV. (*Note:* By a "satisfactory" briquette is meant one with no cracks around the edge.)

The figures in Table IV show that the time for which the briquette is held at the maximum pressure cannot be reduced to zero even under the most favorable circumstances. Assuming a machine which operates at lower pressures than 10,000 lb. per sq. in. a pressing time of at least 20 sec. is required. With 15 sec. for the release of pressure added to this, it is apparent that 35 sec.

TABLE IV

PRESSING TIME TO GIVE A SATISFACTORY BRIQUETTE*

| Temperature | Pressure | | | |
|---|---|---|---|---|
| | 2000 | 5000 | 10,000 | 20,000 |
| | Time, sec. | | | |
| 180°C. | 76 | 45 | 35 | 20 |
| 200°C. | 55 | 40 | 20 | 15 |
| 220°C. | 45 | 30 | 15 | 10 |
| 240°C. | 30 | 20 | 10 | 5 |

** 15 gm. of 2 in. wheat straw (4% moisture).*

is required for the whole pressing operation. To this must be added the time required for filling the mold with loose straw and that required for removing the briquette.

*Effect of Amount of Straw per Briquette*

All the briquettes considered so far have contained 15 gm. (1/2 oz.) of straw each. It is probable that their size is related to their strength, other things being equal, and to test this the weight of straw used was varied from 10 to 30 gm. The temperature was kept constant at 220°C., 2 in. straw used, and the pressure maintained for 30 sec. The quality of the product was judged to be Good, Fair, or Poor according to whether the briquettes showed no cracks, slight cracks, or considerable cracks around the edge. In general, only those designated "Good" would be acceptable.

Table V shows that under the conditions used in the present work there is an upper limit to the amount of straw which can be used for each briquette. With an increasing weight of straw the briquettes become thicker and the gases generated have more difficulty in escaping. However, a very slow release of pressure enabled "good" briquettes to be made with even 30 gm. of material.

*Effect of Moisture Content of Straw*

Under the atmospheric conditions prevailing during these experiments, the moisture content of the straw was stable at 4%. A systematic study was not

TABLE V

EFFECT OF INCREASING AMOUNT OF STRAW PER BRIQUETTE

| Pressure, lb./sq. in. | Weight of straw per briquette in grams | | | | |
|---|---|---|---|---|---|
| | 10 | 15 | 20 | 25 | 30 |
| 2000 | Fair | Fair | Fair | Poor | Poor |
| 5000 | Fair | Fair | Fair | Fair | Fair |
| 10,000 | Fair | Good | Good | Fair | Fair |
| 20,000 | Good | Good | Good | Good | Fair |

made of the effect of different percentages, but several briquettes were made with 10% moisture and with oven-dry straw. There was little or no difference between the 4% moisture and oven-dry material, but with 10% moisture there was excessive steam which made a very slow release of pressure on the mold quite important. With a sufficiently slow release, however, satisfactory briquettes could be made. It is probable that with more than 10% moisture the molding operation would be considerably slowed down, but since an increased pressing time may not be a disadvantage either mechanically or economically, a study of the effect of different degrees of moisture in the straw was considered to be unwarranted at this stage.

*Efficiency of Briquetting Operation*

The foregoing work has shown that from the mechanical point of view quite satisfactory briquettes can be made from wheat straw by heating and pressing. Whether or not they can be made economically poses another question, and while no attempt has been made to answer it here, a consideration has been given to the energy involved during the briquetting process.

The energy required to make a briquette is made up of the mechanical work plus the heat necessary to raise the straw to the required temperature. The energy which may be obtained from it on burning is the calorific value.

The mechanical work may be obtained by plotting the change of volume against the applied pressure, and is equal to:

$$\int_{v_1}^{v_2} P \cdot dv.$$

The value of this integral is most simply obtained by measuring the area under the curve and this was done for 15 gm. of 2 in. straw at 220°C. The curve is shown in Fig. 5, which is also an illustration of the way in which the volume of a briquette changes with pressure. The work performed in making a single 15 gm. briquette proves to be about 190 ft–lb., and it is seen from the curve that owing to the very small volume change, only a very small increase in work results from an increase in pressure from 10,000 to 20,000 lb. per sq. in. Since 15 gm. of straw require 190 ft–lb. work, 1 ton of straw requires 12,160,000 ft–lb. This, expressed as heat, equals 14,800 B.t.u.'s.

The heat necessary to raise the temperature of 1 ton of straw approximately 200°C. is the weight times the specific heat, which for straw is approximately 0.33. On the basis of 4% moisture, the total amount of heat required is 260,000 B.t.u.'s. Therefore, the total energy (expressed as heat) required to make 1 ton of briquettes is 274,800 B.t.u.'s.

The calorific value of straw was determined with a Parr Calorimeter, and averaged 7100 B.t.u.'s per lb. (oven-dry basis). This compares with 7000 B.t.u.'s given by Leslie (15) and 6980 B.t.u.'s given by Rodgers (22). Using the figure 7000 B.t.u.'s per lb., the heat which may be obtained from a ton of wheat straw

is 14,000,000 B.t.u.'s. Thus only about 2% of the available energy is required to make the briquettes. It should be noted that these figures do not take into account the heat required to maintain the losses by radiation from the briquetting machine, but even with this taken into account, the process would appear to be economically sound as far as the energy balance is concerned. The above

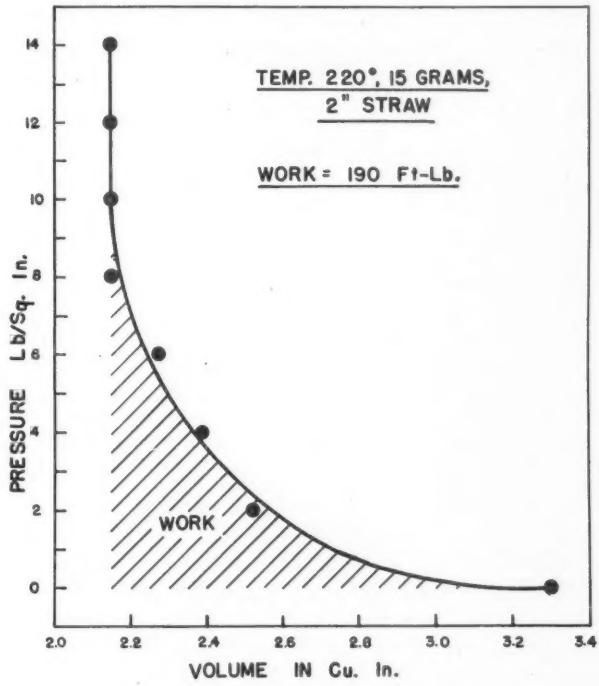


FIG. 5. *Pressure–volume relationship.*

figures may be compared with those of Leslie which he calculated for the "Presto-log" process. In this case a ton of wheat straw logs having a heating value of 14 million B.t.u.'s required the expenditure of 300 to 400 thousand B.t.u.'s.

It has been calculated that 274,000 B.t.u.'s of energy are required to produce a ton of briquettes, and this figure may be divided into mechanical and heat energy. There are two extreme possibilities: (1) that all the heat is supplied by mechanical friction, and (2) that none of it is supplied by friction, it being produced by an auxiliary furnace burning some of the product. The second possibility would certainly not be achieved in practice and it is probable that with a commercial machine running normally, the first condition, i.e., the temperature rise produced by mechanical friction, is the one which would tend to prevail. The importance of knowing whether the heat will be supplied by

friction or by an auxiliary furnace is made apparent by considering the output of briquettes which could be expected from a fixed input of mechanical energy, such as a 40 H.P. motor. Operating for eight hours at 35% efficiency it would produce about 1 ton in the first case, and almost 20 tons in the second. Consequently it would appear that the success or failure of a commercial portable briquetting machine depends to a great extent on a knowledge of the frictional heat produced by that particular machine, and this can only be determined with a full scale machine running at its normal operating speed.

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# DETERMINATION OF THE CHARACTERISTICS OF A FLAME PHOTOMETER AND EFFECTS OF INTERFERING SUBSTANCES[1]

By Frances H. Elliott

## Abstract

A method for finding the best flame for use in a Perkin–Elmer flame photometer is described. A comparison of reports on the effects of five interfering substances present in biological materials and three acids used in the preparation of biological materials has been made and reveals that similar flame photometers burning the same fuel differ in their response to the same substance. Owing to the different responses to interfering substances, it is important to test the validity of every step, such as extent of dilution, in the preparation of a biological material, even though it has been found satisfactory in another laboratory.

## Introduction

In recent years, flame photometers have found widespread use in the analysis of sodium and potassium in biological materials. Among the papers that have described the workings and usefulness of flame photometers, several (3, 5, 11) have emphasized the errors involved in their use. These arise mainly from interfering substances and from unstable conditions, such as fluctuation in air and gas pressures. In the present paper, techniques for recognizing and avoiding errors in the operation of a flame photometer, using the direct method of analysis, will be reported.

## A Method for Finding the Optimum Settings for Air and Gas Pressures

Many publications (2, 6, 7, 9, 11, 12) describe the principle of the flame photometer and general methods of operation. In all papers the need for a steady flame is stressed, and many authors have mentioned the air and gas pressures which they have found suitable to give a steady flame; but these optimum settings are not the same for different machines. In fact, specific settings of air and gas pressure are meaningless for defining a flame in the Perkin–Elmer instrument, the machine which has been used in our studies. In this apparatus, one can only know the air and gas pressures as these gases leave their tanks and not the pressures in the burner. The air pressure, after the air leaves its gauge, is altered by the capillary tube in the atomizer, and the gas pressure by the needle valve in the burner.

Mosher *et al.* (8) have described a method for obtaining steady readings in the Beckman flame photometer. They found that, as the oxygen pressure to

[1] *Manuscript received October 30, 1950.*
*Contribution from the Allan Memorial Institute and the Department of Psychiatry. McGill University, Montreal, Que. This work was supported in part by funds from the National Research Council of Canada.*

the flame was increased, the intensity of light from an atomized sodium or potassium solution increased to a maximum and then decreased. With a given flame, increasing the rate of atomization also increased the intensity of light to a maximum. In the vicinity of these peaks, changes in pressure of oxygen, fuel gas, and rate of atomization cause relatively slight changes in light intensity and, hence, dial readings are most steady and accurate. It was pointed out that the best settings for oxygen and gas pressure and atomization rate vary with the kind of gas used, the atomizer used, and the metal to be analyzed. These settings must be rechecked periodically.

We could not find the relationships between air, gas pressures, and the rates of atomization as Mosher did, because of the differences between the Beckman and Perkin–Elmer photometers. In the Perkin–Elmer apparatus, compressed air is used both for atomizing the solution and for supplying oxygen to the flame. Thus the air pressure to the flame cannot be adjusted independently of the rate of atomization, expecially when a nonadjustable glass atomizer is used. In order to study the effects of the pressures of air and fuel gas and of the rate of atomization independently, light intensities of atomized solutions were measured at given air pressures as the gas pressure was changed, the air–gas ratio being recorded as a measure of the relative supply of oxygen to the flame. The rate of atomization was varied by changing the air pressure and was measured by timing the flow of 5 cc. of water through the atomizer funnel.

It is readily observed that the color of a flame obtained with the lowest air-gas ratio at a given air pressure is green-blue and changes by decreasing the gas pressure to a purple feathery appearance at the highest air–gas ratio just before the flame blows out. The needle valve in the burner was adjusted so that a rather high gauge reading for gas pressure was registered. This needle valve adjustment was not changed throughout the experiments; and at this adjustment of the needle valve, the air–gas ratio for the greenest blue flame was 1.9, and for the most purple flame about 3.3 when the air pressure was varied from 10 to 17 lb. per sq. in. The temperatures of the blue green and purple flames were measured by an optical pyrometer using a platinum wire heated in the center of the burner. For all air pressures used the purple flame (air/gas 3.3) had a temperature of approximately 1170°C. ± 10° and the green blue flame (air/gas 1.9) 1080°C. ± 10°.

In Fig. 1, the light intensities from sodium solutions 1.5 and 0.8 m.e. per liter obtained using 8-17 lb. per sq. in. of air pressure are plotted against air–gas ratios. It can be seen that the light intensity from both sodium solutions increases with increasing air–gas ratio (or temperature) and reaches a maximum at an air–gas ratio approaching 3. Thus an air–gas ratio of 3, which yields a purple flame, gives the most steady readings. Light intensities measured as described above are subject to variations in the amplifying system. A repetition of these tests on another day showed the same relative relationships, but the absolute values were lower.

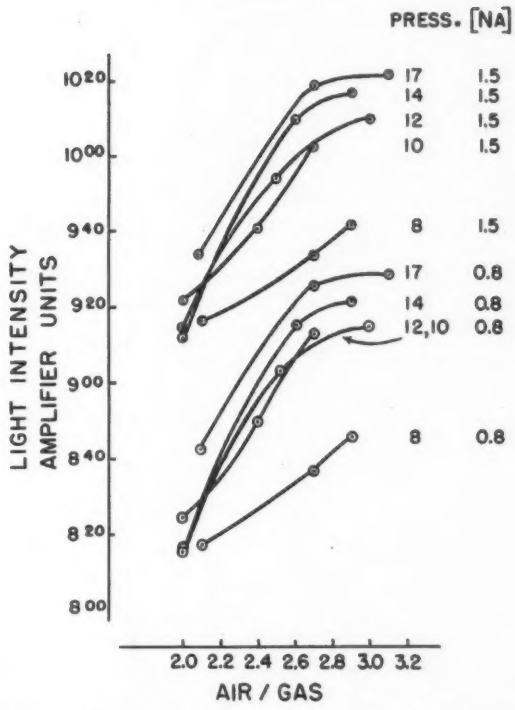



FIG. 1. *The effect of adjustment of the flame (air/gas) on the light intensity of sodium solutions. Air pressures are in lb. per sq. in. and concentrations of sodium are m.e. per liter. The light intensity measurement is the amplification of the current from the photo cell necessary to give a galvanometer reading of 100. In our apparatus, 60 units on the fine gain amplifier dial are equivalent to 1 on the coarse gain dial. Hence a reading of 8 (coarse), 60 (fine) is the same as 9 (coarse), 00 (fine). The greater gain readings indicate lower amplification and, hence, higher light intensity. Therefore, the gain readings are directly proportional to light intensity.*

The effect of changing the rate of atomization by changing the air pressure on the maximum light intensity is shown in the graphs of Fig. 2. There are no maxima in these curves. Hence, on the basis of these curves, within this range of rates of atomization no rate is optimal. However, at air pressures of 17 or over, the drain of the atomizer is overtaxed. An air pressure of 12 lb. per sq. in. has been found satisfactory.

In practice the optimum setting of the air and gas pressures for sodium determinations is obtained as follows: the flame is lighted with the air pressure set at 12 lb. per sq. in. While atomizing a sodium solution of about 1.0 m.e. per liter the gas pressure is adjusted until a maximum dial reading is obtained.

In Fig. 3 the light intensities from potassium solutions 2.5, 2.0, 1.0, and 0.5 m.e. per liter are plotted against air–gas ratios. With potassium solutions the

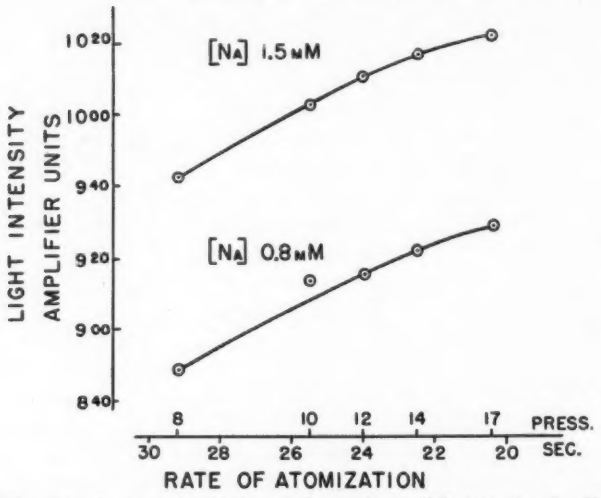
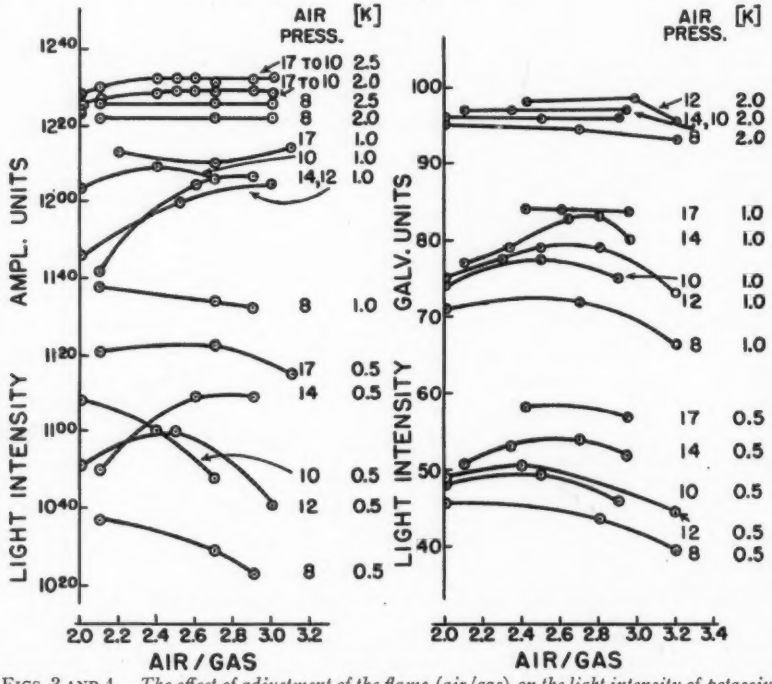



FIG. 2. *The effect of rate of atomization on the maximum light intensity of sodium solutions at indicated air pressures. Along the ordinate are marked the rates of atomization of 5 ml. of solution in seconds and the air pressures which produced those rates.*




FIGS. 3 AND 4. *The effect of adjustment of the flame (air/gas) on the light intensity of potassium solutions. In Fig. 3 the light intensity is measured in amplifier units. In Fig. 4 the light intensity is measured in galvanometer units. (See text.) Air pressures are in lb. per sq. in. and concentrations of potassium are m.e. per liter.*

effect of the air–gas ratio (or temperature of the flame) on the light intensity of atomized solutions varies with the concentration of potassium and, especially at the two lower concentrations, with the rate of atomization. A solution containing 2.5 m.e. potassium per liter produces a light only slightly affected by either rate of atomization or temperature. This is also true with a 2.0 m.e. per liter solution, which yields about the same light intensity as the 2.5 m.e. per liter solution. The flame is apparently saturated with potassium at these concentrations. Hence, concentrations of potassium greater than 2 m.e. per liter cannot be measured accurately.

To eliminate the possible effects of variations in the amplifying system, light intensities in Fig. 4 were measured not by the amplification of the light from the photo cell but by the galvanometer readings relative to light from a solution of potassium 2.5 m.e. per liter giving a dial reading of 100, since the light from the latter solution is unaffected by changes in the conditions under study.

As seen in Fig. 4, the maximum light intensity is obtained at an air–gas ratio of less than 2.8, i.e. at a lower temperature than for the sodium solutions. As the air pressure is decreased, this maximum effect shifts to a lower air–gas ratio or to a cooler flame. It may also be noted that the effect of a change in air–gas ratio is not so marked as with sodium solutions.

Again, as with sodium solutions, there are no peaks in the curves showing the effect of change in rate of atomization on light intensity (Fig. 5). Hence, there is no optimal air pressure. However, the higher air pressures are better to use since a decrease in rate of atomization at the lower air pressures shifts

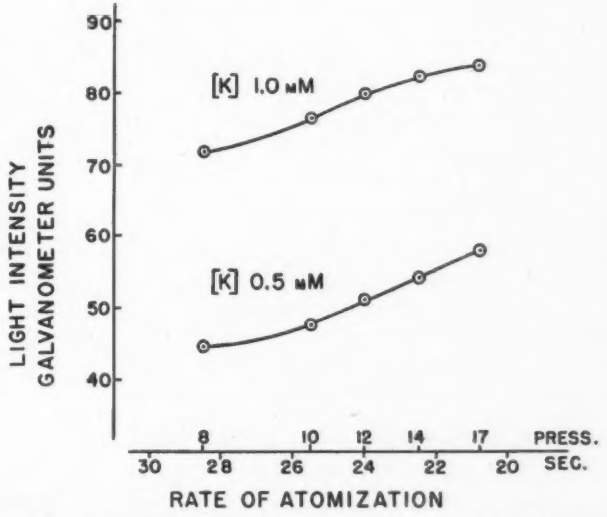



FIG. 5. *The effect of rate of atomization on the maximum light intensity of potassium solutions at indicated air pressures; the ordinate as in Fig. 2.*

the maximum effect of the air–gas ratio. Moreover, a slight change in either air or gas pressure affects the air–gas ratio least when the higher air pressures are used.

In practice, to obtain the optimum air and gas pressure settings for the determination of potassium, the flame is lighted with an air pressure setting of 12 or 14 lb. per sq. in., and while atomizing a solution of potassium of about 1 m.e. per liter strength, the gas pressure is adjusted until a maximum dial reading is obtained.

The rate of atomization is affected not only by the air pressure, but also by small particles collecting in the atomizer. Fig. 5 shows that a slight decrease in rate of atomization, 1 sec. per 5 cc., decreases the galvanometer reading by approximately one and one-half units. Fine particles readily collect in the small opening of the atomizer and change the rate slightly. Hence it is necessary to test the rate often. For best results, it is necessary to filter all solutions through ash-free filter paper, such as Whatman No. 40.

The value of the above flame adjustments has been tested in practice. Successive galvanometer readings for sodium solutions vary very slightly when the proper flame is used. If the flame is changed to a blue color, the variations in galvanometer readings become larger and troublesome. Readjustment to the proper purple flame restores the steadiness of the readings.

The above adjustments of the air and gas pressures would not necessarily be best for other flame photometers, nor even for ours if the burner or atomizer were changed, but the same principles for finding the optimal settings could be applied to all instruments.

Even with the optimum adjustment of the flame, one cannot rely on a standard curve remaining constant. A complete standard curve is used only to obtain a rough estimate of the value of an unknown solution. For accurate determinations, many standard solutions are prepared, in steps of 0.05 m.e. per liter and the sample value calculated from the nearest standards.

## Interfering Substances

It is well recognized that each flame photometer is unique in that adjustments for optimum operation are unique, but it seems to be assumed that uniform interfering effects are obtained with all photometers if the same gas is used for fuel. This assumption is not justified. It has been pointed out that when acetylene is used, interfering substances often caused an increased intensity of light of the metal analyzed, whereas the opposite is true when natural gas or propane is used (6).

In Figs. 6 and 7 are plotted the per cent errors in the determination of sodium and potassium caused by eight substances, phosphoric acid, potassium, sodium, ammonia, and urea (the main interfering substances in urine and blood), hydrochloric acid, nitric acid, and sulphuric acid (acids most commonly used

in wet ashing procedures). The errors plotted are the results in the analyses of solutions containing 2 m.e. per liter of potassium or 3.5 m.e. per liter of sodium, found by Parks *et al.* (11), by Berry *et al.* (3), and in our laboratory. Parks *et al.* used a Perkin–Elmer model 18 burning natural gas. Berry *et al.* used a similar machine burning propane. Our machine is a Perkin–Elmer Model 52A burning propane.

The difference in the results obtained with these three machines is striking. In our photometer, ammonia, phosphoric acid, and hydrochloric acid interfere to a greater extent in the potassium determination than in the sodium deter-

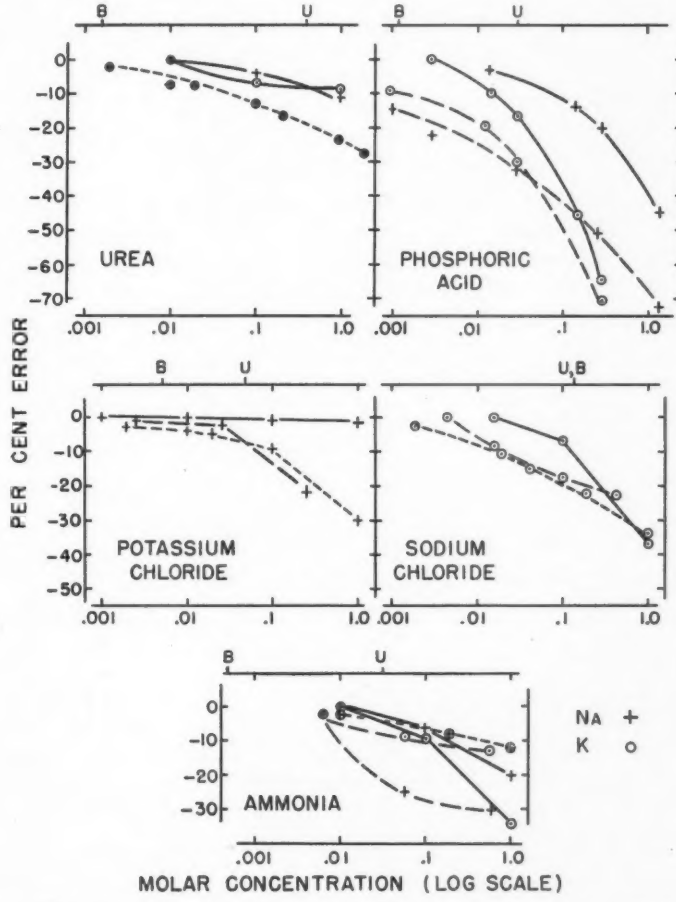



FIG. 6. *The errors caused by five interfering substances present in blood and urine in the determination of sodium 3.5 m.e per liter and potassium 2 m.e. per liter.*
*The results of Parks et al. are given by broken lines; of Berry et al., by short dash lines; and of the author, by solid lines. Sodium values are indicated by +; and potassium values by o.*

mination. Berry *et al.* found that each interfering substance caused about the same error in the determination of both metals. Parks *et al.* found that hydrochloric and sulphuric acids interfered to a greater extent in the potassium than in the sodium determination, and ammonia caused a greater interference in the sodium than in the potassium determination. Crismon (5) using a Perkin–Elmer photometer burning propane, reported a much greater interference in the sodium than in potassium determination due to phosphate ions.

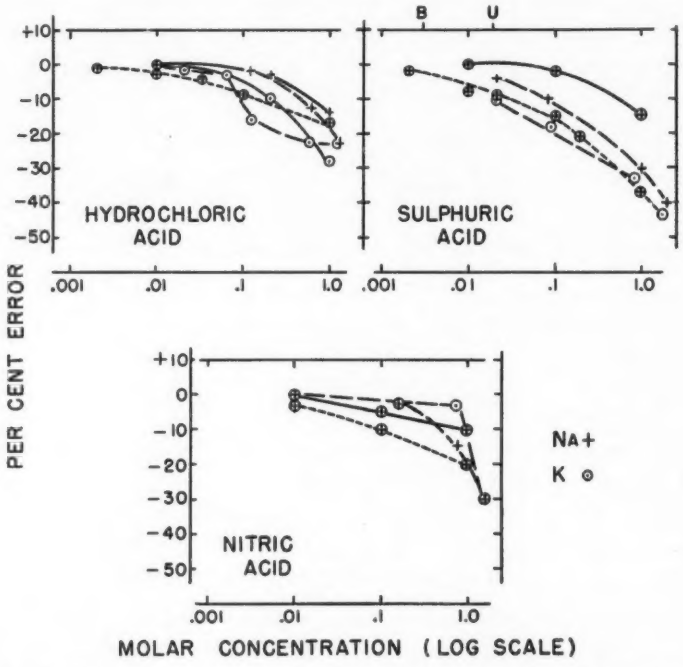



FIG. 7. *The errors caused by three acids in the determination of sodium and potassium. See Legend of Fig. 6.*

In preparing a biological material for analysis in the flame photometer, a most important consideration is elimination of the effects of interfering substances present in the sample itself or added as a deproteinizing or a wet-ashing agent. Since, as shown above, interference effects differ from apparatus to apparatus, a method of preparing a biological material might not be suitable for analysis in all flame photometers.

With some biological fluids such as blood, urine, and saliva, some workers find it necessary only to dilute the biological material prior to flame photometry. The dilutions required to eliminate the effects of interferences must be found for each machine. On the other hand, if the dilution required to eliminate

the effects of interferences is greater than that required for the analysis, then interfering substances whose effects cannot be eliminated by dilution have to be added to standard solutions in the same concentration as in the sample.

*Preparation of Urine*

Hald (7) has suggested diluting urine 1/20 with water prior to analysis for potassium and 1/40 for sodium determinations. In our experience, a greater dilution is necessary to avoid interference in determining potassium. A group of 41 samples of urine were analyzed for potassium; each sample was diluted 1/20 and 1/50. In another group of 41 samples, the dilutions used were 1/50 and 1/100. In the first group the average potassium concentration was 50.4 ± 2.62 m.e. per liter with a mean difference of 1.9 m.e. per liter between the duplicate values; in the second group, the average value was 62.6 m.e. ± 1.46 m.e. per liter, with a mean difference of 1.16 m.e. per liter between duplicate values. With the sodium determinations on the same urines, the mean differences between duplicate values were the same for all dilutions. Thus dilutions of 1/50 or 1/100 are preferable to 1/20 for analysis of potassium in urine, but the low dilution is satisfactory for sodium in our apparatus.

That urine should be diluted 1/50 to avoid interferences in the potassium determination in our apparatus is illustrated in the following results. Solutions were prepared containing potassium 2 m.e. per liter, sulphuric acid, 0.02 *M*; phosphoric acid, 0.03 *M*; ammonium chloride, 0.034 *M*, and urea, 0.42 *M*. Four other solutions were prepared containing potassium 2 m.e. per liter and 1/10, 1/20, 1/50, 1/100 of the above concentrations of interfering substances. These are the main interfering substances in urine and are present in a so-called "average" urine in the concentrations listed above. These concentrations are indicated by "U" in Figs. 6 and 7. Corresponding solutions containing sodium 3.5 m.e. per liter were also prepared. The solutions were then analyzed for potassium and sodium in the flame photometer with the following results:

| Dilution of interfering substances | Potassium 2 m.e. per liter % error | Sodium 3.5 m.e. per liter % error |
|---|---|---|
| 1/1 | −25 | −3.4 |
| 1/10 | − 8 | 0 |
| 1/20 | − 3 | 0 |
| 1/50 | 0 | 0 |
| 1/100 | 0 | 0 |

These errors are approximately equal to the sum of the errors estimated from Figs. 6 and 7 (our values). Hence it can be predicted from Fig. 6 that Parks *et al.* should dilute urine much more than 1/30 to determine potassium and sodium accurately to avoid the interfering effect of $PO_4$ in his apparatus, and that Berry *et al.* should dilute urine 1/200 to avoid the effect of urea.

Shapiro and Hoagland (13) used 1/100 and 1/300 dilutions for sodium and potassium analyses respectively. These authors removed phosphate from 39 samples of urine and found that the values of sodium and potassium were not altered by the removal of phosphate. This work was done in answer to Crismon's paper (5) in which he reported large errors due to phosphate interference.

Domingo and Klyne (6), using a flame photometer burning acetylene, reported that potassium values of urine diluted 1/25 or 1/50 with water are considerably higher than those obtained by the cobalti–nitrite method of Abul Fadl (1), but when the urine is dry ashed previous to flame photometry, tolerable agreement (within ± 10%) in the values is obtained by the two methods. There is a possibility that the ashing was not necessary for obtaining the correct potassium value, but rather that the ashing resulted in a loss of potassium. The solution of the ashed urine was used both for the chemical analysis, and for flame photometry. Cholak and Hubbard (4) report that when an organic matrix of a biological material must be destroyed by dry ashing, lower values for sodium, potassium, and calcium are obtained than by wet ashing. Our experience with dry ashing urine is in agreement with Cholak and Hubbard's.

*The Preparation of Blood Serum*

As indicated in Figs. 6 and 7, the concentrations of the interfering substances in blood serum indicated by "B", with the exception of sodium, are lower than in urine, "U". It is reasonable, therefore, that a dilution of only 1/10 as suggested by Hald (7) has been found satisfactory by us for the analysis of potassium in blood serum. The blood serum may be diluted with 0.9% sodium chloride to avoid the precipitation of protein. For comparisons with these solutions, potassium standards containing 0.9% sodium chloride are used. In our machine, it is possible to determine potassium in blood serum diluted 1/10 with water using, for comparison, standards that contain only potassium chloride. The aqueous solution of blood serum is slightly cloudy, but when the solution is freshly prepared, it will not clog the atomizer. Reference to Fig. 6 shows that 14 m.e. of sodium per liter, which is the concentration of sodium in blood serum diluted 1/10, does not interfere in the determination of potassium in our machine. However, it can be seen that this concentration of sodium would cause an 8-10% error with the photometers of Parks *et al.* and Berry *et al.* Parks *et al.* should dilute serum 1/23 to avoid this error, and Berry *et al.*, 1/77. Since this latter dilution is too great for accurate determination of potassium, sodium chloride would have to be added to the potassium standards and to the diluted blood serum in proper concentration (about 0.9%) for accurate results in the photometer of Berry *et al.*

Domingo and Klyne (6) diluted blood serum 1/10, using a solution of sodium chloride containing 330 mgm. sodium per 100 ml. (0.84% sodium chloride solution) and used potassium standards containing the same concentration of sodium chloride.

Overman and Davis (10) deproteinized blood serum with trichloroacetic acid, using an amount which gave a final concentration of acid of 2.4%. On our machine, 5% trichloroacetic acid results in a 25% error in the analysis of potassium in blood serum and an 11% error in the analysis of sodium.

*Wet Ashing*

Overman and Davis (10) state that the acids required in wet ashing and the salts resulting from their neutralization interfere to an extent in their apparatus

that renders impossible the use of these ashing procedures for preliminary treatment of biological materials.

However, Hald (7) and Cholak and Hubbard (4) have used wet ashing procedures successfully prior to flame photometry.

It is of interest to note the concentrations of acids present in the final solutions for analysis of food stuffs, whole blood, and feces after wet ashing, according to the directions of Hald (7).

| | HCl | $H_2SO_4$ | For analysis of |
|---|---|---|---|
| Food stuff | 0.24*M* | 0.04*M* | K |
| | 0.5 | 0.08 | Na |
| Feces | 0.24 | 0.04 | K |
| | 1.2 | 0.2 | Na |
| Whole blood | | 0.01 | Na |

By referring to Fig. 7, one can see that hydrochloric acid in the concentrations suggested by Hald would have marked interfering effects in all three photometers, and sulphuric acid in all concentrations recommended would affect the analytical results in the apparatus of Parks *et al.* and Berry *et al.* The interfering effects of these acids would have to be compensated for by adding them in the same concentration to the standard potassium and sodium solutions used.

It has been suggested that the differences in the interfering effects obtained in an acetylene flame from those in a propane or natural gas flame are due to the higher temperature of the acetylene flame. Thus it seemed possible that the differences in interfering effects observed above might be due to differences in the temperature of the flames used and indeed, the possibility of getting different effects in one's own apparatus, if the temperature were varied, presented itself. The interfering effects of six concentrations of phosphoric acid from 1.5 to 0.03 *M* were tested on potassium solutions of concentrations of 1.5 to 0.5 m.e. per liter. Many adjustments of the flame were used, using air pressures from 8 to 17 lb. per sq. in. Only when the flame was changed from the "purple" (1170°C.) to the most green blue color (1080°C.) was there a detectable difference in interference with any concentration of phosphoric acid, and this difference was only about twice the experimental error (0.10 m.e. potassium per liter). The higher interference was obtained at the highest temperature. The temperatures obtainable with different burners might be far different from 1080°–1170°C.

*This comparison of the results of many workers emphasizes that the interfering effects of foreign substances vary from machine to machine, and this fact makes it necessary for every analyst to test, on his own flame photometer, the suitability of a procedure for preliminary treatment of a biological material, even though it is found suitable by another worker using the same type of flame photometer and gas fuel.*

*Analysis of Biological Solutions*

Analyses of urine, blood serum, gastric juice, saliva, and milk for potassium; and of urine, blood serum, and saliva for sodium have been success-

fully carried out in our flame photometer. With all these fluids, the effects of interfering substances have been eliminated by dilution with water as follows:

| | Dilution | | For analysis of |
|---|---|---|---|
| Urine | 1/50 | 1/100 | K and Na |
| Blood serum | 1/100 | | Na |
| | 1/10 | 1/20 | K |
| Saliva | 1/25 | | K and Na |
| Milk | 1/50 | 1/100 | K |
| Gastric juice | 1/10 | 1/20 | K |

The dilutions were selected which gave the maximum value for the metal analyzed, and from which known added amounts of sodium and potassium were recovered completely. Two dilutions of urine are always measured because its composition is very variable.

*The Accuracy of the Method*

If interfering effects are eliminated, the proper flame settings found, and a large series of standards used, potassium and sodium values can be obtained that are accurate to a value that corresponds to one galvanometer unit, that is to 1/100 × the strength of the maximum standard used. Using a 0.5 m.e. per liter solution of potassium or sodium as maximum standard, an absolute error (A) of 0.005 m.e. per liter is obtained. Thus, the absolute error in the original solution analyzed will be absolute error A × dilution factor. Hence, potassium in blood serum, for example, can be determined correctly to 0.005 × 10 = 0.05 m.e. per liter or 0.005 × 20 = 0.1 m.e. per liter. The maximum percentage error with a serum containing 4 m.e. of potassium per liter, that is low normal, would be 2.5% (0.1 × 100/4).

We have found that the magnitude of the absolute error obtained with the diluted solutions analyzed is usually no greater than 0.02 m.e. per liter and never greater than 0.04 m.e. per liter even when an unsteady flame is used, providing many standard solutions varying in strength by only 0.05 m.e. per liter are used for comparison.

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# DISSIMILATION OF GLUCOSE BY YEAST AT POISED HYDROGEN ION CONCENTRATIONS[1]

By A. C. Neish and A. C. Blackwood


## Abstract

A distiller's yeast was grown anaerobically in media containing yeast extract (0.5%) and glucose (5%), the pH being controlled within ± 0.05 pH units by automatic addition of ammonium hydroxide. The sugar was almost completely fermented in 14–47 hours over the range pH 2.4 to 7.4 but very little at pH 2.0 or 8.0. When sodium hydroxide is used in place of ammonia the fermentation is completed at pH 8.0 but not at pH 8.2. If the initial glucose concentration is increased to 25% about 80–85% of the sugar is fermented in five to seven days in the range pH 4.0–pH 6.4 but at pH 7.0 only half of it is utilized. The yield of glycerol, based on the sugar fermented, is increased by increasing the initial glucose concentration or the pH or by using ammonia in place of sodium hydroxide. The highest yield of glycerol obtained was 29% by weight of the sugar fermented. Aeration increases the rate of fermentation but causes lower yields of alcohol and glycerol.


## Introduction

Numerous papers have been published on the growth and metabolism of yeast. Some of these deal with the effect of pH on the fermentation of glucose, using buffers to obtain control. Some workers have used large quantities of yeast, hence they have been observing primarily the effect of pH on fermentation while others using relatively small inocula have been measuring the effect on growth as well.

When comparatively large amounts of yeast are used it has been found (4) that fermentations require about the same time to reach completion at any pH in the range 4.0–8.5. In strongly alkaline media fermentation is inhibited more than respiration (16).

When small inocula are used, and the yeast grown on synthetic media, the optimum growth occurs at pH 3.4 to 3.9 although there is an initial lag period which is much less pronounced at a higher pH (15). This optimum varies with different strains and may be as high as pH 6.0 for some yeasts (7). If yeast is grown in a medium containing large amounts of glucose and no attempt is made to control the pH it may fall as low as pH 2.2 before growth ceases (5). Additions of organic acids to a medium containing sucrose has shown that the hydrogen ion concentration must be increased to pH 2.3–2.7 before growth of yeast is prevented (17). Growth in alkaline media is very poor above pH 7.7–8.0 although tolerance to alkali is increased if large amounts of inoculum are used (3).

It is well known that the amount of glycerol produced by yeast is considerably increased if alkaline fermentations are carried out (14). With some

[1] *Manuscript received May 29, 1950.*
*Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan. Issued as paper No. 102 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2315.*

strains of yeast, glycerol may be obtained in yields up to 25% of the sugar fermented (2). It is possible that even better yields might be obtained under more closely controlled conditions.

The present paper reports an investigation on the effect of pH on the yeast fermentation. It differs from previous work in that a monitor capable of giving control within ± 0.05 pH units was employed. Comparatively small inocula were used and the effects of varying the sugar concentration, the oxygen tension, and the neutralizing agent were studied at a number of closely controlled hydrogen ion concentrations.

## Experimental

*Preparation of Inoculum and Media*

The same strain of distiller's yeast was used in all experiments. It was obtained from Dr. Elizabeth McCoy, University of Wisconsin, and numbered Y-2 in our collection. The inoculum was grown at 30°C. in a medium containing 1.0% glucose and 0.5% Difco yeast extract. Ten milliliters of a 20 hr. old inoculum was used for 250 ml. of medium in each fermentation. The medium was prepared by mixing three volumes of a glucose solution with one volume of 2.5% yeast extract and one volume of a salts solution. Each of these solutions was sterilized separately and cooled before mixing. The salts solution contained $KH_2PO_4$(0.25%), $K_2HPO_4$ (0.20%), $MgSO_4.7H_2O$ (0.10%), $FeSO_4$ (0.025%), $CaCl_2$ (0.05%), and NaCl (0.10%). It is a suspension which must be shaken well before aliquoting. The concentration of glucose was chosen to give a final concentration of 5–25% as desired.

*Control of Fermentations*

The pH was controlled by automatic addition of sodium hydroxide or ammonium hydroxide solutions using the same type of flask and apparatus as previously described (13). The glass electrodes were sterilized by soaking overnight in 0.1% mercuric chloride, rinsing with sterile water, and then exposing to ultraviolet light (13). Anaerobic conditions were obtained by continuously bubbling purified nitrogen (9) through the fermenting solution while carbon dioxide–free air was used to obtain aerobic conditions. The temperature was maintained at 30°C.± 0.25°.

*Analysis of Fermentation Solutions*

When the fermentations were finished, as shown by cessation of glucose utilization or alkali addition, the solutions were acidified with hydrochloric acid and swept out with the gas stream in order to remove all of the carbon dioxide. The carbon dioxide was determined gravimetrically, by absorption in Caroxite. The solution was cleared with zinc hydroxide as described previously (9) but some changes were made in the analytical methods. The ethanol was determined as before but the glycerol was estimated by a colorimetric method based on measurement of the formaldehyde formed on periodate

oxidation (8). The organic acids were determined by partition chromatography on silica (10) after extraction from the acidified fermentation solution by ether. A considerable amount of water is extracted from alcoholic solutions, hence the extract was made to a definite volume with water, after evaporation of the ether, rather than attempting to add enough *tert*-amyl alcohol to the ether extract to give a solution of the acids in an organic solvent, as before (10). This allows the acids to be concentrated 10-fold during the extraction. An aliquot of the aqueous solution of the extracted acids (0.5 ml.) was analyzed on a silica-water column (10) packed with the upper 15% of it left dry to absorb the water in the sample (12). 2, 3-Butanediol and acetoin were determined by procedures found useful with bacterial fermentations (9), at first, but these were changed to more sensitive and specific methods during the investigations. Acetoin was finally estimated in distillates by the reaction with alkaline creatine and alpha-naphthol (18) while 2, 3-butanediol was determined by measurement of the acetaldehyde formed on periodate oxidation. The acetaldehyde was separated from the fermentation solution during periodate oxidation by microdiffusion into bisulphite (19), and estimated by measurement of the color produced on reaction with piperazine and sodium nitroprusside (1). Methods based on oxidation to diacetyl, such as that of Hooreman (6), are probably more specific but attempts to use them were abandoned when it was found that the yield of diacetyl was different for different isomers of 2, 3-butanediol. For example D-(*levo*)-2, 3-butanediol gave a yield of 72 + 3% while the (*meso-dextro*)-2, 3-butanediol produced by *Aerobacter aerogenes* gave a yield of 81 ± 2% under the same conditions. Since the 2, 3-butanediol produced by yeast is predominately D-(*levo*)-2, 3-butanediol (11) results obtained by these methods are too low when *meso*-2, 3-butanediol is used as the standard. The specificity of methods based on periodate oxidation can be greatly increased by partition chromatography (12).

## Results and Discussions

The results of a fairly extensive investigation into the effect of pH on the anaerobic dissimilation of dilute glucose solutions (5%) are shown in Table I. Ammonium hydroxide was used as the neutralizing agent. This fermentation was rather insensitive to the hydrogen ion concentration in the range pH 2.4 to 7.4, considering that there are 100,000 times as many hydrogen ions at the lower pH. However, there was only a small amount of sugar fermented at pH 2.0 (8%) or 8.0 (19.6%), most of this being fermented in the first 10 hr. after inoculation. Compared to the bacteria which have been studied in this way (13) yeast is characterized by a somewhat greater sensitivity to alkali and a tolerance for at least 1000 times as great a hydrogen ion concentration. The rate of fermentation shows a rather broad optimum from pH 4.0 to 6.6. As expected from previous work the yield of glycerol and acetic acid increased with increase of the pH. The highest yield of glycerol obtained was 17.9% of the weight of the sugar fermented (pH 7.4).

TABLE I

ANAEROBIC DISSIMILATION OF DILUTE GLUCOSE SOLUTIONS BY YEAST WITH AUTOMATIC pH CONTROL USING AMMONIUM HYDROXIDE

The fermentations were run in a medium containing 5% glucose, the pH being controlled by automatic addition of ammonium hydroxide

| Product | Millimoles of product per 100 millimoles of glucose fermented | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH 2.4 | pH 3.0 | pH 3.4 | pH 4.0 | pH 5.0 | pH 5.6 | pH 6.0 | pH 6.6 | pH 7.0 | pH 7.4 | pH 7.6 |
| 2, 3-Butanediol | 0.76 | 0.75 | 0.43 | 0.48 | 0.46 | 0.45 | 0.53 | 0.45 | 0.45 | 0.51 | 0.68 |
| Acetoin | Nil | Nil | 0.02 | Nil | Nil | Nil | Nil | Nil | 0.07 | 0.07 | 0.19 |
| Ethanol | 169.7 | 171.5 | 171.8 | 177.0 | 172.6 | 164.0 | 160.5 | 149.2 | 149.5 | 136.9 | 129.9 |
| Glycerol | 8.13 | 6.16 | 6.56 | 6.60 | 7.82 | 13.0 | 16.2 | 19.2 | 22.2 | 35.0 | 32.3 |
| Butyric acid | 0.16 | 0.13 | 0.18 | 0.32 | 0.25 | 0.16 | 0.36 | 0.44 | 0.25 | 0.45 | 0.21 |
| Acetic acid | 0.98 | 0.52 | 0.46 | 0.69 | 0.84 | 1.95 | 4.03 | 6.14 | 8.68 | 15.1 | 15.1 |
| Formic acid | 0.27 | 0.36 | 0.26 | 0.42 | 0.63 | 0.09 | 0.82 | 0.14 | 0.35 | 0.75 | 0.49 |
| Succinic acid | 0.62 | 0.53 | 0.48 | 0.26 | 0.32 | 0.52 | 0.49 | 0.52 | 0.23 | 0.51 | 0.68 |
| Lactic acid | 0.59 | 0.82 | 0.77 | 0.38 | 0.47 | 1.29 | 1.63 | 1.44 | 1.93 | 1.26 | 1.37 |
| Carbon dioxide | 181.2 | 180.8 | 176.8 | 189.8 | 187.6 | 174.8 | 177.0 | 164.8 | 161.0 | 160.0 | 148.5 |
| Glucose carbon assimilated | 15.3 | 12.4 | – | 16.1 | 14.0 | – | 12.4 | 16.5 | – | – | – |
| Fermentation time, hr. | 41 | 29 | 25 | 14½ | 17½ | 14½ | 15½ | 14½ | 35 | 47 | 25 |
| % Glucose fermented | 98.4 | 98.5 | 98.1 | 97.0 | 96.5 | 98.2 | 98.0 | 98.5 | 98.3 | 96.9 | 60.3 |
| % Carbon recovered | 95.1 | 93.8 | 91.2 | 98.0 | 96.3 | 92.5 | 96.4 | 93.1 | 92.5 | 97.3 | 91.3 |
| O/R Index | 1.04 | 1.03 | 1.01 | 1.05 | 1.06 | 1.02 | 1.05 | 1.03 | 1.00 | 1.03 | 1.01 |

An investigation of the effect of using sodium hydroxide as the neutralizing agent has shown that the fermentation can be carried to completion in somewhat more alkaline media (see Table II). However this does not result in any better yields of glycerol. Ammonia was slightly better than sodium hydroxide for production of glycerol, in these experiments.

TABLE II

ANAEROBIC DISSIMILATION OF DILUTE GLUCOSE SOLUTIONS BY YEAST WITH AUTOMATIC pH CONTROL USING SODIUM HYDROXIDE

Conditions as stated for Table I except that sodium hydroxide was used in place of ammonium hydroxide

| Product | Millimoles of product per 100 millimoles of glucose fermented | | | | | |
|---|---|---|---|---|---|---|
| | pH 5.0 | pH 6.0 | pH 7.0 | pH 7.6 | pH 8.0 | pH 8.2 |
| 2, 3-Butanediol | 0.40 | 0.39 | 0.38 | 0.33 | 0.36 | 0.33 |
| Acetoin | nil | nil | 0.01 | 0.01 | 0.01 | 0.03 |
| Ethanol | 177.0 | 165.9 | 168.8 | 148.0 | 142.5 | 136.2 |
| Glycerol | 6.10 | 10.4 | 11.3 | 25.1 | 29.4 | 31.3 |
| Butyric acid | 0.27 | 0.39 | 0.04 | 0.35 | 0.26 | 0.43 |
| Acetic acid | 1.20 | 4.27 | 4.30 | 9.16 | 9.31 | 10.5 |
| Formic acid | 0.14 | 0.46 | 0.41 | 0.43 | 0.52 | 0.25 |
| Succinic acid | 1.16 | 1.14 | 0.51 | 0.43 | 0.45 | 0.43 |
| Lactic acid | 1.53 | 1.73 | 0.94 | 0.87 | 0.94 | 0.92 |
| Carbon dioxide | 187.0 | 178.0 | 170.5 | 167.8 | 160.2 | 160.1 |
| Fermentation time, hr. | 14 | 16 | 15½ | 32 | 46 | 48 |
| % Glucose fermented | 99.1 | 98.5 | 99.9 | 98.1 | 98.0 | 55.7 |
| % Carbon recovered | 96.0 | 94.0 | 91.2 | 94.1 | 93.1 | 94.5 |
| O/R index | 1.04 | 1.03 | 1.01 | 1.04 | 1.02 | 1.05 |

The difference in the yields of 2, 3-butanediol between Tables I and II is due to a change in the analytical methods. The results in Table I, obtained by the older methods (9), are undoubtedly high but are reported since they at least set a maximum value. The figures in Table II for 2, 3-butanediol and acetoin were obtained by the more sensitive and specific methods described above. It is of interest that the yields of these compounds are insensitive to variation of the hydrogen ion concentration over a range which markedly affects their production by bacteria (13).

The fermentation of more concentrated glucose solutions was investigated in order to see how much glucose could be fermented at a favorable pH. It was found that about 22–23 gm. of glucose per 100 ml. of medium could be utilized (see Table III), in the range pH 4.0–6.4, both aerobically and anaerobically.

TABLE III

DISSIMILATION OF CONCENTRATED GLUCOSE SOLUTION BY YEAST WITH AUTOMATIC pH CONTROL USING AMMONIUM HYDROXIDE

The fermentations were run in a medium containing 23–25% glucose. Anaerobic conditions were maintained by bubbling a stream of purified nitrogen through the medium. Aerobic fermentations were given about 13 volumes of air per hour

| | Gm. per 100 ml. of medium | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Anaerobic fermentation | | | | | | Aerobic fermentation | | |
| | pH 4.0 | pH 5.0 | pH 5.6 | pH 6.0 | pH 6.4 | pH 7.0 | pH 6.6* | pH 7.0 | pH 7.4 |
| Glucose at start | 25.3 | 24.4 | 26.6 | 23.8 | 25.8 | 25.2 | 23.7 | 22.6 | 22.9 |
| Glucose at end | 3.7 | 3.3 | 4.3 | 2.6 | 4.0 | 13.1 | 0.7 | 0.6 | 12.9 |
| Glucose fermented | 21.6 | 21.1 | 22.3 | 21.2 | 21.8 | 12.1 | 23.0 | 22.0 | 10.0 |
| Glycerol formed | 1.11 | 1.34 | 2.51 | 2.46 | 3.05 | 3.50 | 1.35 | 1.37 | 2.08 |
| Ethanol formed | 10.1 | 10.2 | 9.90 | 8.89 | 9.59 | 4.45 | 8.10 | 7.84 | 3.33 |
| Fermentation time, days† | 4 | 5 | 7 | 7 | 7 | 5 | 2 | 2 | 2 |
| Time actually run, days | 6 | 6 | 9 | 8 | 9 | 9 | 3 | 2 | 2 |

* *Contained 1.09% acetic acid—not determined in the others.*
† *Determined by daily analysis for glucose and from the rate of alkali addition.*

The fermentations are more rapid under aerobic conditions but the yields of glycerol and ethanol are not as good, possibly because of respiration. The most interesting result of using a high initial glucose concentration is the increased yield of glycerol relative to the ethanol. This can best be seen by reference to figures in Table IV, which have been calculated from the data in Tables I and III. It is obvious that the yield of glycerol is increased by raising either the initial concentration of glucose or the pH. By using concentrated solutions, yields of glycerol were obtained up to 29% of the sugar fermented (see Table

III, anaerobic fermentation at pH 7.0). This is a good yield considering the yeast used has not been selected for, or adapted to, glycerol production.

Whenever high yields of glycerol are obtained the fermentations are slow and incomplete. It seems to be necessary to inhibit the ethanol-producing mechanism of yeast before glycerol can be obtained in good quantities. This may be done by raising the pH, as has been known for some time, or by in-

TABLE IV

EFFECT OF THE INITIAL GLUCOSE CONCENTRATION ON THE RATIO OF GLYCEROL TO ETHANOL FORMED UNDER ANAEROBIC CONDITIONS

| pH of medium | Ratio of glycerol/ethanol by weight | |
|---|---|---|
| | 5% Glucose | 23–25% Glucose |
| 4.0 | 0.067 | 0.110 |
| 5.0 | 0.091 | 0.131 |
| 5.6 | 0.158 | 0.254 |
| 6.0 | 0.202 | 0.277 |
| 6.4 | ..... | 0.318 |
| 6.6 | 0.257 | ..... |
| 7.0 | 0.297 | 0.786 |
| 7.4 | 0.512 | ..... |

creasing the concentration of glucose. It can be seen from Table III that fairly close control of the pH is important when concentrated glucose solutions are fermented. The result obtained at pH 6.4 is interesting since most of the sugar was fermented to give a solution containing over 9.5% ethanol and 3.0% glycerol. The difficulties of recovering glycerol from such a solution on a commercial scale may not be as great as in some of the patented fermentation processes. However ethanol is still the major product, the yield of glycerol being only 14% of the weight of the sugar fermented. A small amount of aeration might increase the rate of fermentation without much decrease in the yield of glycerol and ethanol. Good yields of products might also be obtained by fermenting a concentrated glucose solution at pH 7.0 for two days and then allowing the pH to fall to 6.4 so the rest of the sugar may ferment.

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